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ANATOMY OF THE HYPOCOTYL AND ROOTS OF DAUCUS CAROTA[1]

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INTRODUCTION

The cultivated carrot (*Daucus carota* L.) is a biennial plant of the Umbelliferae family. The first year of its growth the hypocotyl and the upper portion of the primary root become fleshy. The stem is very short during this first year, but, during the second growing season, a flower stalk extending to a height of 2 to 4 feet is produced. In the study here reported an attempt has been made to follow the anatomical development of the root and hypocotyl from the seedling stage to edible maturity, that is, to the end of the first season.

REVIEW OF LITERATURE

The anatomy of the hypocotyl and roots of the carrot apparently has never been studied in detail. A few studies, however, have been made of various phases of the anatomy of several plants belonging to the Umbelliferae family. Gérard (*3*)[2] reported, in a general way, the transition from root to stem structure in several plants of the Umbelliferae, but he did not mention the carrot. Van Tieghem (*7*) and Van Tieghem and Douliot (*8*) described the origin of lateral roots from the primary one in several plants of this family. Apparently, however, they made no specific study of the carrot in this regard. Van Tieghem (*7*) also noted the oil ducts in several members of the Umbelliferae. Trécul (*6*) described briefly the characteristic vessels found in a number of plants of the same family. He also mentioned the secretory canals found in several plants of this family including the carrot. Courchet (*2*) reported on some of the gross anatomical characteristics of the Umbelliferae. Warning (*9*) recently reported studies on the anatomy of the vegetative organs of the parsnip. From her studies it seems that in many respects the carrot and parsnip are quite similar in certain anatomical characters but strikingly different in others.

MATERIAL AND METHODS

Plants at successive stages of development were used in this study. The plants used were mainly of the Chantenay variety and were grown in the greenhouses of the Ohio Agricultural Experiment Station. Plants 8 to 10 days old proved most satisfactory for the study of the primary body of the hypocotyl and roots. From this stage to maturity numerous other specimens were secured throughout the growth of the plants.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 564.

The specimens were killed, dehydrated, and embedded in paraffin by the butyl alcohol method. The transverse and longitudinal sections were cut 10 to 12μ in thickness, mounted serially, and stained, usually with safranine and fast green.

GROSS MORPHOLOGY OF THE SEEDLING

The initial development of the seedling is shown in the rupturing of the seed coat by the development of the hypocotyl and the radicle. The elongation of the inverted V-shaped hypocotyl brings the cotyledons out of the soil and the hypocotyl into a vertical position. In the greenhouse at 60° to 70° F. this entire process is completed in 5 to 6 days. The cotyledons are oblanceolate and radiate-veined, and the petioles are 5 to 6 mm. in length. As is the case in many seedlings, the cotyledons are undiverged at their bases and form a cotyledonary tube in which the epicotyl is located. In the carrot this tube is relatively short, extending only 30 to 40 μ above the cotyledonary node.

In the carrot seedling the upper extremity of the primary root may be located by the smaller diameter of the root in contrast to the hypocotyl above it. Furthermore, most lateral roots arise from the primary root although often a few roots may be seen developing from the lower portion of the hypocotyl of the seedling. The upper extremity of the hypocotyl is terminated by the cotyledonary node. The hypocotyl is usually 15 to 25 mm. in length; the actual length depends largely on the moisture, soil, temperature, and light conditions under which the plants are grown.

ANATOMY OF THE SEEDLING

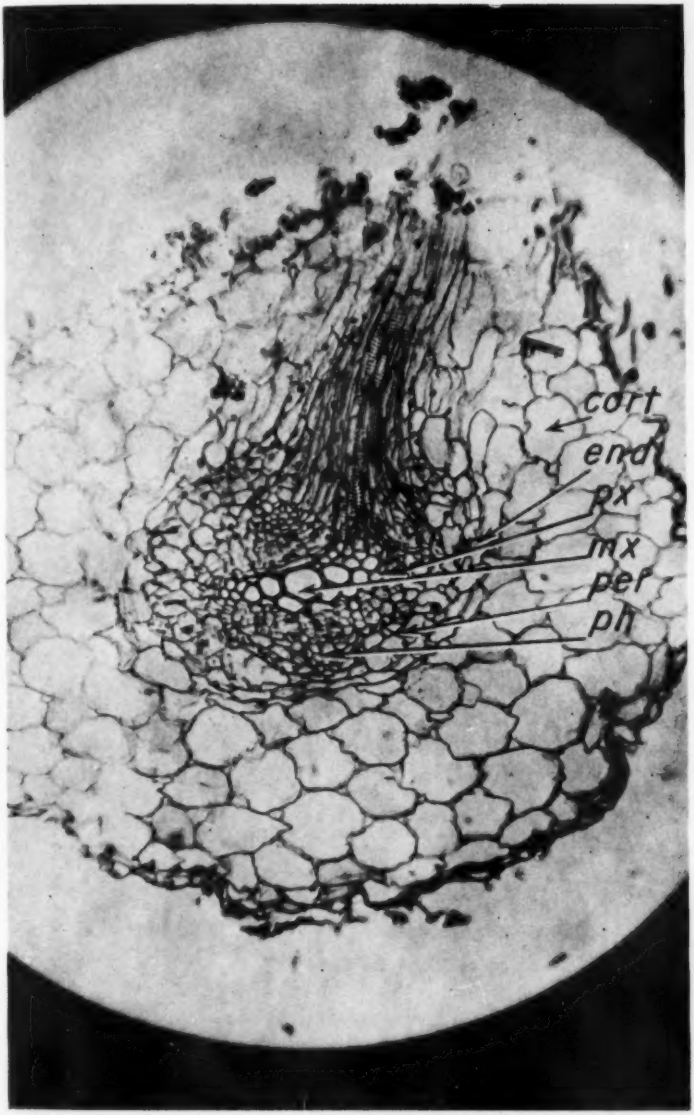
THE PRIMARY ROOT

The structure of the primary root of the carrot seedling as it appears 8 to 10 days after the seed is planted is not at all unusual. The xylem and phloem are radially arranged in the primary body of the young root (pl. 1). The xylem is diarch and is composed of four to six protoxylem elements which are abaxially arranged with respect to the six to eight metaxylem cells. No pith was found in the root. The phloem is separated from the diarch xylem by three to four layers of parenchyma, one of which will form the cambium. The primary phloem is composed of sieve tubes, companion cells, and parenchyma.

In the young primary root the pericycle is composed of one or two layers of rather irregularly shaped parenchymatous cells. This sheath of pericycle surrounds the phloem and abuts the two protoxylem points. Lateral roots originate from the pericycle, usually near a protoxylem point but not opposite it (pl. 1). The endodermal cells vary in size and shape, but as a whole they are larger than those of the pericycle. The Casparian thickenings (of the endodermis) are seen as somewhat inconspicuous dots or strips along the radial cell walls. The cortex is composed of five to seven layers of irregularly shaped parenchymatous cells. In this parenchyma the intercellular spaces are comparatively large. The epidermis is one layer thick and is the first tissue to differentiate from the promeristem, followed closely by the cortex.

THE YOUNG HYPOCOTYL

As previously stated, the hypocotyl of the carrot seedling is usually 15 to 25 mm. in length, and lateral roots may arise from the lower portion of it. The transition from root to stem structure occurs in



Transverse section of young primary root. Note diarch xylem, radial arrangement of xylem and phloem, and origin of lateral root: *cort*, Cortex; *end*, endodermis; *px*, protoxylem; *mx*, metaxylem; *per*, pericycle; *ph*, phloem. × 280.

the upper one-seventh or one-eighth of the hypocotyl (2 to 3 mm.); thus the larger part of the hypocotyl is rootlike in anatomical structure. The anatomical description already given of the young primary root is applicable to six-sevenths to seven-eights of the hypocotyl.

The Transition Zone

Below are given the anatomical changes which occur in the transition from root to stem structure in the primary body, beginning with the

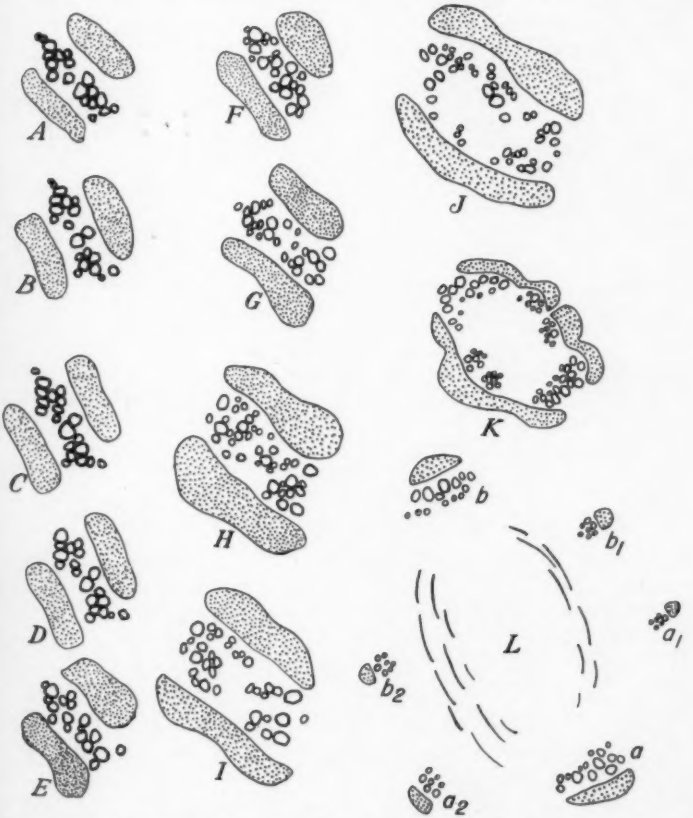


FIGURE 1.—*A–K*, Partly diagrammatic representation of the primary vascular system of the transition zone in series from the upper part of the hypocotyl into the cotyledons. *L*, *a* and *b*, midribs of the two cotyledons; a_1, a_2, and b_1, b_2, lateral bundles of the two cotyledons respectively. The phloem is stippled in all cases.

upper limit of rootlike structure of the hypocotyl and continuing through the upper hypocotyl to the base of the cotyledons, where the transition is complete. A reorientation of the primary body is first evident 2 to 3 mm. from the cotyledonary node (fig. 1, *A*). Here a

small pith is present, and the protoxylem points are not so distinct (fig. 1 *A*, *B*, *C*) as below that region. As a higher level is reached, the primary xylem elements are more and more separate from each other and are more numerous (fig. 1, *C*, *D*, *E*, *F*, *G*). The protoxylem becomes more adaxially arranged, and the metaxylem is differentiated toward the outside or to an abaxial position. The primary phloem shows little change until a position only 1 to 1½ mm. from the cotyledonary node is reached (fig. 1, *H*). The phloem then is seen to be differentiated more and more around the xylem as a higher plane is cut (fig. 1, *I*, *J*, *K*) until it almost encircles it.

About 1 mm. from the cotyledonary node the primary xylem elements form six groups (fig. 1, *J*, *K*). Here the phloem is organized around these xylem groups, and a collateral arrangement of the xylem and phloem results (fig. 1, *K*, *L*). This is usually at the cotyledonary node. The bundles are endarch as well as collateral here where they diverge into the cotyledons in most carrot seedlings, although in some cases the transition is completed in the bases of the cotyledons. The larger bundles (fig. 1, *L*, *a* and *b*) make up the midribs of the two cotyledons and the smaller bundles (fig. 1, *L*, a_1, a_2, b_1, and b_2) make up the lateral bundles of the two cotyledons respectively. For example a_1 and a_2 represent lateral bundles which diverge into the same cotyledon as does the midrib represented by *a* (fig. 1, *L*).

It may be noted that, as in salsify (*4*), dahlia (*5*), and many other plants, the primary vascular bundles of the root and hypocotyl are not continuous with those of the epicotyl. The primary bundles of the hypoctyl diverge entirely into the two cotyledons, as already described. The endarch collateral bundles of the epicotyl are differentiated soon after the primary bundles are formed in the hypocotyl. In the older plant, of course, bundles in the root, hypocotyl, and stem are continuous.

ONTOGENY OF THE HYPOCOTYL AND ROOTS

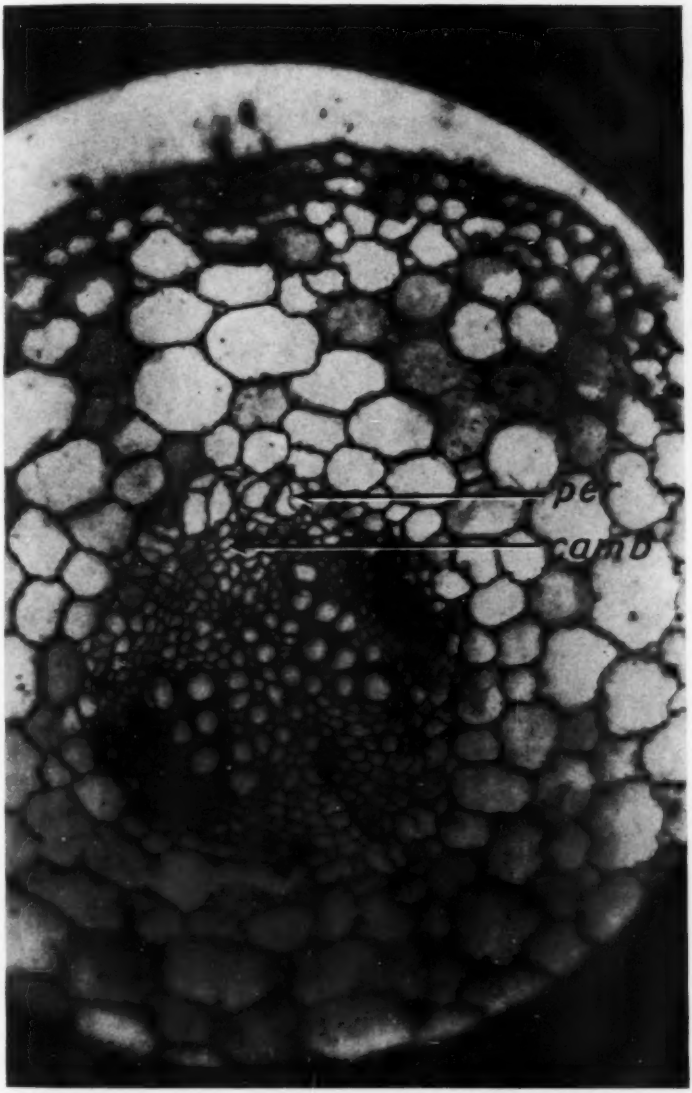
THE HYPOCOTYL AND UPPER TAPROOT

The edible portion of the carrot is largely hypocotyl and varying amounts of the upper taproot, the relative amount of taproot depending somewhat on the horticultural variety. Thus, with the exception of the few millimeters of transition zone at the upper part of the hypocotyl, the enlarged edible portion of the mature carrot is anatomically of root structure.

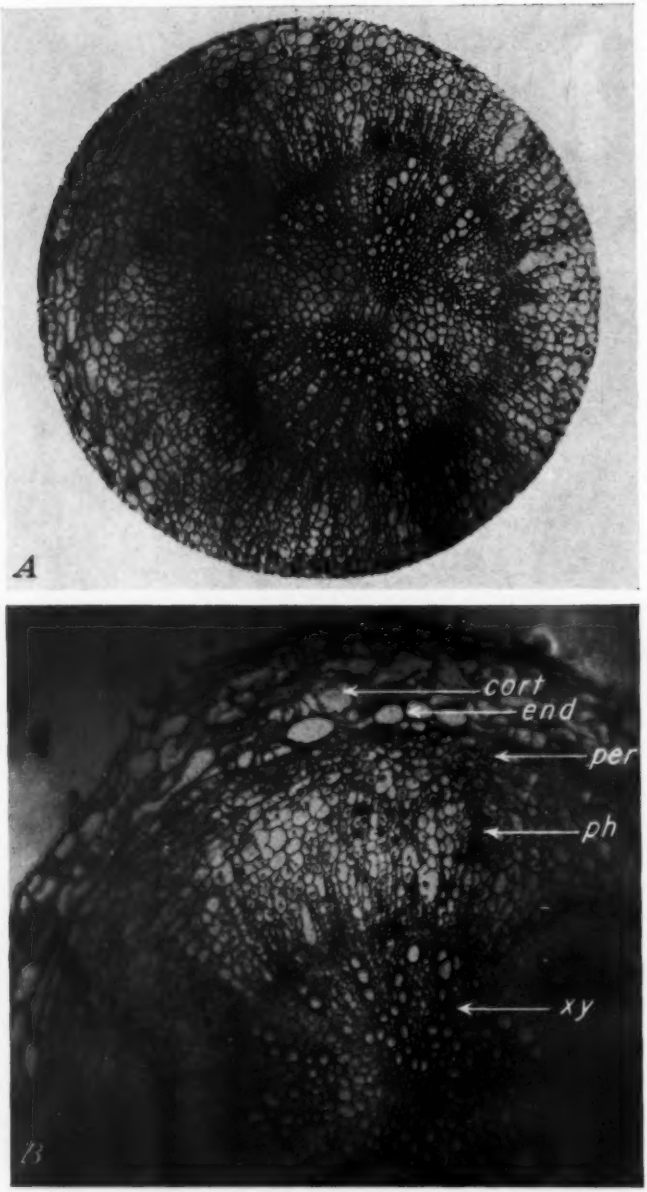
Early Secondary Thickening

The first evidence of secondary thickening is noted in the procambium or parenchyma between the primary xylem and phloem groups. These cells first elongate axially; then tangential division takes place. Thus the primary cambium originates, and secondary xylem and phloem is developed in the usual manner (pl. 2). Tracheae, tracheids, and considerable parenchyma are laid down in the xylem; meanwhile sieve tubes, companion cells, and parenchyma are developed in the phloem.

Almost simultaneous with the development of the primary cambium near the phloem poles is the division of the pericycle parenchyma opposite the protoxylem points. After only a few divisions of these



Early secondary thickening of hypocotyl. Note activity of pericycle and interfascicular cambium: *per*, Pericycle; *camb*, cambium. × 300.



Transverse section through upper hypocotyl showing growth and development of the various tissues. *A*, Central tissues at about the time the cortex is shed. × 115. *B*, Sector showing enlargement of hypocotyl just before the cortex is shed: *cort*, Cortex; *end*, endodermis; *per*, pericycle; *ph*, phloem; *xy*, xylem. × 155.

cells, an interfascicular cambium is formed from the pericycle which becomes continuous with the primary cambium between the xylem and phloem previously described (pl. 2). This interfascicular cambium does not form immediately a cylinder of secondary vascular tissue but for some time forms parenchyma only. In this way two wide parenchymatous rays are developed opposite the protoxylem points (pls. 2 and 3).

Shortly after the formation of the first secondary thickening a small amount of radial cell division takes place in the endodermis; thus this tissue remains one cell in thickness, but keeps pace with the enlargement of the stele. The cortical parenchyma thickens very little, although there is a small amount of radial cell division here during the early stages of secondary thickening.

Period of Further Enlargement

The primary xylem, especially the protoxylem, becomes so distorted as secondary growth continues that it is soon hardly recognizable. However, the original relative positions of the points may easily be located since the primary medullary rays of parenchyma are always opposite them (pl. 3). During the growth in diameter of the fleshy root and the hypocotyl of the carrot, the xylem, as well as the phloem parenchyma, remains meristematic, although to a lesser degree than the pericycle. The division and enlargement of the parenchyma is especially active in about the upper one-fourth of the hypocotyl (pl. 3). In fact, the pithlike center of the upper portion of the mature edible carrot is almost entirely xylem parenchyma, although there is a small amount of pith in the transition zone, which, as previously described, extends only a few millimeters below the cotyledonary node. Both *A* and *B* of plate 3 were cut through the lower part of the transition zone.

Eventually the cambium opposite the protoxylem points lays down tracheae, tracheids, and considerable parenchyma in the xylem and a relatively small number of seive tubes, companion cells, and parenchyma in the phloem. Thus, while the hypocotyl and enlarging root are only 3 to 5 mm. in diameter, there is a continuous ring of secondary vascular tissues. Several rays of secondary xylem parenchyma are usually present, across which the interfascicular cambium is active. These rays in the secondary vascular tissue are sometimes opposite the protoxylem points, although more often they are at angles of 30° to 50° with the original protoxylem position (pl. 3). At right angles to the primary xylem the cambium is extremely active. At these two opposite positions xylem and phloem are developed with a considerable amount of parenchyma. Here the phloem extends almost to the endodermis, indicating that the pericycle is relatively inactive at these places. However, opposite the original protoxylem points and early parenchymatous rays the phloem is relatively narrow, and the pericycle is quite active.

The endodermal cells continue to divide radially as the fleshy part of the carrot develops; thus the endodermis remains one layer thick (pl. 3, *B*).

Very little cell division takes place in the cortex. Because of the pressure from the growing stele the cortical cells become flattened tangentially, and the cortex is shed when the fleshy root and hypocotyl

are 3 to 5 mm. in diameter (pl. 3, *B*). Just before the cortex is shed, a dermatogen layer begins to form from the pericyclic cells just inside the endodermis (pl. 3, *B*).

The Mature Structure

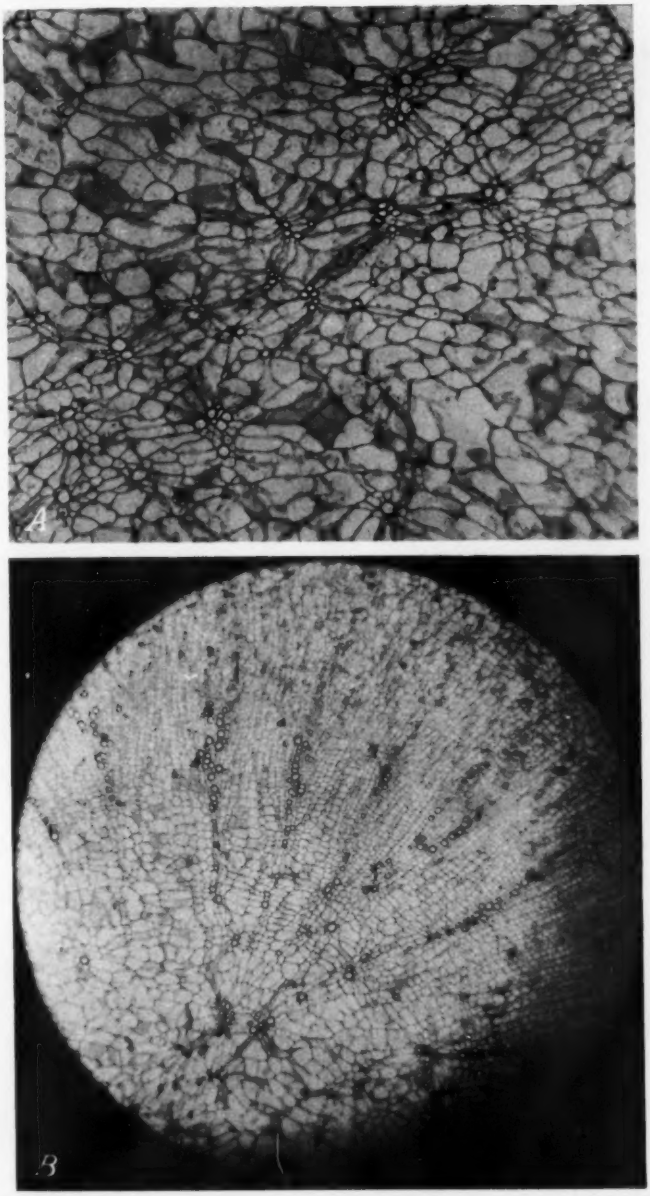
The mature enlarged or fleshy root and hypocotyl of *Daucus carota* is composed largely of parenchyma. If one examines a cross section of a mature carrot (enlarged part) with the unaided eye a pithlike center is noted. This is relatively large at the upper part of the hypocotyl and is smaller as a lower region is reached. With the exception of the few millimeters of transition zone in the upper hypocotyl, this pithlike zone consists largely of thin-walled xylem parenchyma and not true pith. At this stage the primary xylem usually cannot be identified since the protoxylem has disintegrated and the metaxylem has become scattered throughout this central area (pls. 4 and 5). This phenomenon is due mainly to the enlargement of the central parenchymatous xylem cells, although some division of these cells occurs (pl. 4, *A*). In the short transition zone there is a true pith composed of thin-walled parenchyma which is surrounded by the primary xylem in the younger plant. The primary xylem, here too, usually loses its identity in the mature structure owing to the enlargement and division of the pith and xylem parenchyma. In the lower root region, where much less enlargement takes place, there is little proliferation of the parenchyma, and the parenchymatous cells become lignified relatively early. The primary xylem remains intact here.

The parenchymatous rays in the xylem of the mature enlarged root and hypocotyl increase in size mainly through cell enlargement, although some cell division occurs (pl. 4, *B*). In the mature structure the two broad bands of parenchyma originally opposite the protoxylem points still persist. The vessels lose much of the ray arrangement noted earlier in the ontogeny because of the activity of the parenchyma surrounding them (pl. 5). The size of the xylem, or "core," in proportion to the thickness of the tissues surrounding it (separated by cambium) varies considerably with the carrot variety. A relatively small core is usually associated with a high-quality variety.

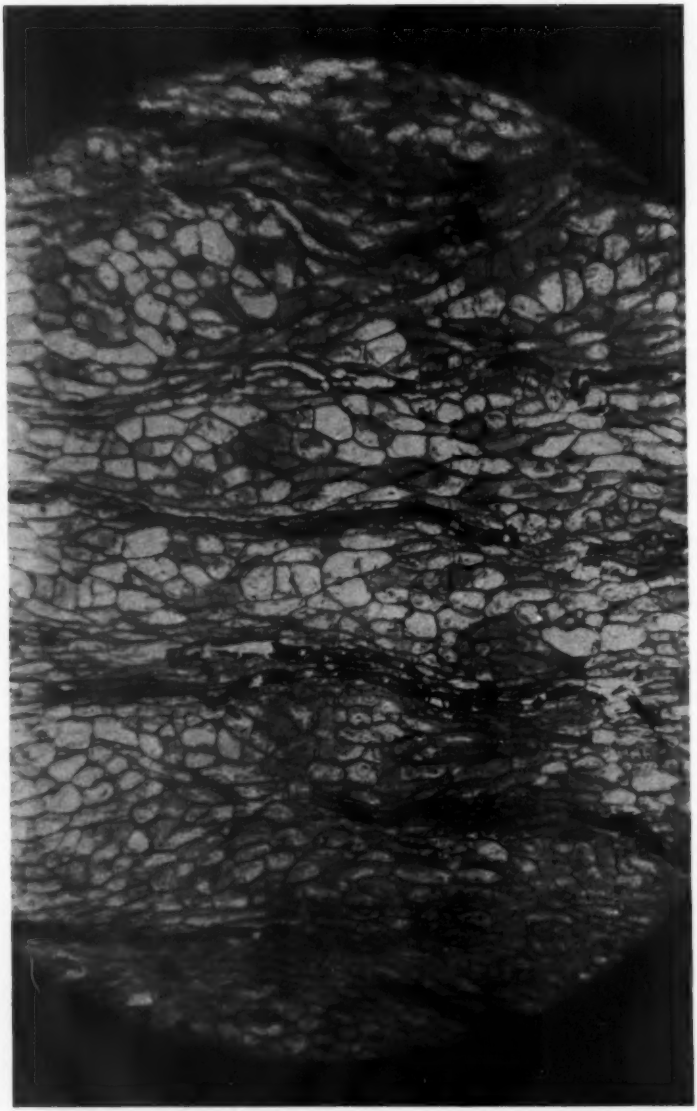
The primary cambium remains active as the edible carrot reaches maturity. The cambial region is composed of four to six rows of thin-walled, compact, rectangular cells, as seen in transverse sections.

The phloem of the mature structure consists of a relatively large amount of parenchyma, although there are also sieve tubes, companion cells, and a few fibers. There are two large groups of phloem located opposite each other and radially arranged with respect to the original primary xylem. The two groups of primary phloem which, earlier in the ontogeny, were located on the outer edge of these large secondary phloem groups, disintegrate until they cannot be located in the mature carrot. These two secondary phloem groups extend to within a few cells of the periderm of pericyclic origin. Oil ducts are also present near the periphery of the phloem (pl. 6, *A*).

The pericycle makes up about a third as much of the region outside of the cambium as the phloem. It is only a few layers in thickness opposite the two large phloem groups described in the preceding paragraph, but in other localities it is much thicker. The pericycle remains meristematic throughout the growing season; both radial and



Transverse sections through central regions of enlarged hypocotyl: *A*, Distortion of primary xylem and enlargement and division of central xylem parenchyma. × 252. *B*, The same as *A*, showing also principal xylem rays, cambium, and phloem. × 84.



Longitudinal section through hypocotyl cut at about the same stage and place as transverse section shown in plate 4, *A*. Note distortion of vessels by growth of parenchyma. × 200.

tangential division of cells takes place (pl. 6, *A*). Oil ducts are present near the periphery of the pericycle. These are quite similar in their development to those described by Trécul (*6*) as characteristic of the Umbelliferae, and also to those of the parsnip as described by Warning (*9*). The periderm, formed from pericyclic cells, is five to eight cells in width (pl. 6, *A*). The phellogen forms cork cells on the outside and phelloderm cells inside. The number of cork cells formed is much greater than the number of phelloderm cells, since the cork cells are constantly sloughed off and replaced.

LATERAL ROOTS

The lateral roots of the carrot originate in the pericycle of the primary or taproot at an angle of about 45° with the primary xylem group (pl. 1). This is also true of roots which originate in the lower hypocotyl since it is rootlike in anatomical structure. There are thus four rows of lateral roots located about 90° apart on the diarch tap root and hypocotyl. Van Tieghem (*7*) cites several plants of the Umbelliferae in which he found that lateral roots arose at a 45° angle to the primary xylem. He states that the lateral roots arise by the division of the pericyclic cells on radii between the vessels and the phloem of the primary root. He notes further that the pericycle opposite the protoxylem points has no "caractère spécial" which prevents the origin of rootlets, and that he does not know the reason for their failure to originate there.

As the lateral root originates, the endodermal cells at the tip of this root elongate and then divide as the root continues to develop; finally the endodermis is ruptured. The lateral roots develop through the cortex by mechanical pushing and also by digestion of cortical cells.

The general structure of the lateral roots is similar to that of the lower primary root. There is relatively less thin-walled parenchyma, however, and more lignified cells. The secondary lateral roots, as well as the tertiary ones, are diarch. Phloem fibers are developed early and are relatively numerous (pl. 6, *B*).

Lateral roots often arise at the periphery of the pericycle during early secondary growth of the primary or taproot and the hypocotyl (pl. 7, *A*) and also after the cortex has disappeared (pl. 7, *B*). Similar phenomena have been observed in the beet by Artschwager (*1*) and in the parsnip by Warning (*9*). In the case of the carrot this type of lateral roots seems to arise always from the pericycle near the place of emergence of the endogenous lateral roots already described. Thus while only one root, which may or may not be permanent, arises endogenously at a specific point, there is often a group of several roots opposite a root trace in the older carrot.

SUMMARY

The carrot seedling has oblanceolate, radiate-veined cotyledons, which are photosynthetic. The epicotyl is enclosed in a short cotyledonary tube. Lateral roots arise from the lower hypocotyl as well as from the primary root.

The transition zone in the seedling is located in the upper part of the hypocotyl and is relatively short. Thus, most of the hypocotyl is

rootlike in anatomical structure. The primary root, as well as the lateral roots, is diarch.

From a morphological viewpoint the enlarged or edible part of the carrot is largely hypocotyl, although the upper taproot is enlarged often also, the relative amounts depending on the horticultural variety.

In the early enlargement of the edible portion of the carrot the cambium and the pericycle are quite active. As the carrot develops further the xylem and phloem parenchyma become meristematic, and there is considerable enlargement especially of the xylem parenchyma. The cortex is shed when the fleshy root and hypocotyl are 3 to 5 mm. in diameter. The cork cambium is then derived from the pericycle. The mature enlarged structure is composed largely of xylem, phloem, and pericyclic parenchyma.

Lateral roots originate in the pericycle and at an angle of about 45° with the primary xylem cylinder. Thus, there are four rows of lateral roots located 90° apart on the diarch taproot and hypocotyl. The secondary, as well as tertiary, lateral roots are diarch. Phloem fibers are developed here early and are relatively numerous.

Lateral roots also often arise at the periphery of the pericycle during early secondary growth and also after the cortex of the hypocotyl has disappeared. This type of lateral root arises near the place of emergence of the endogenous lateral root.

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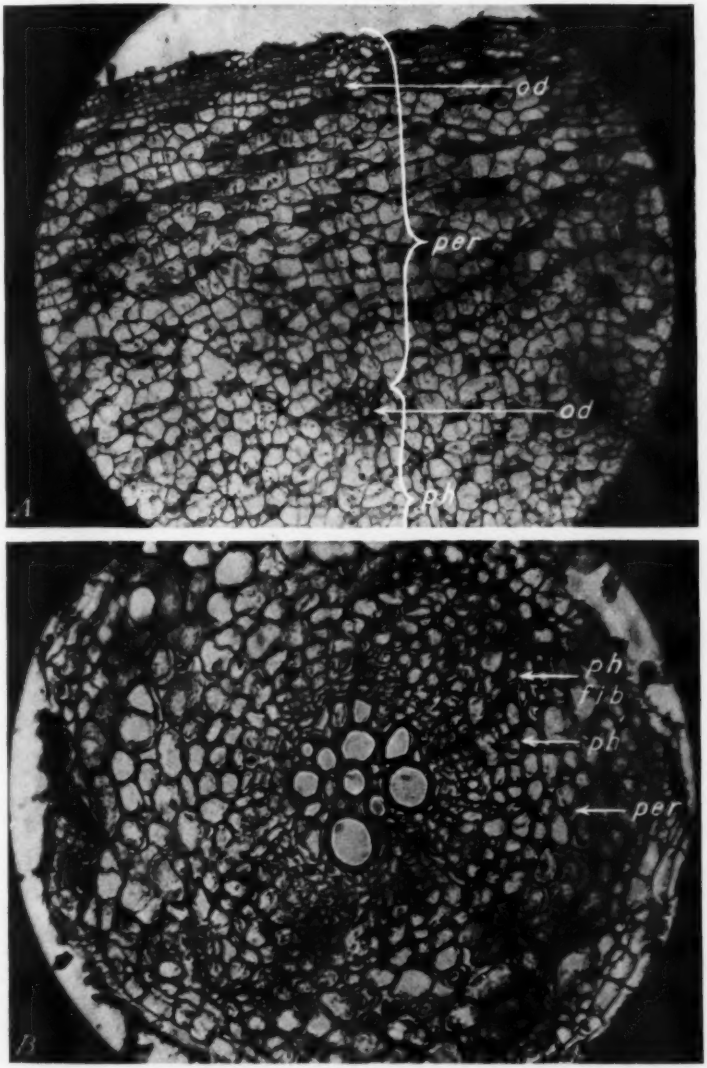
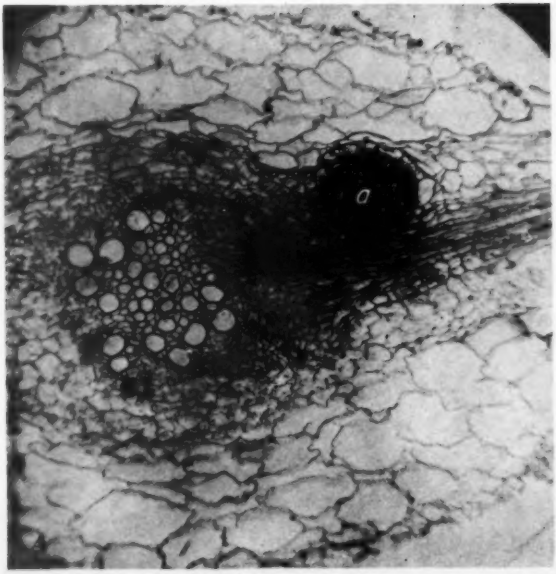
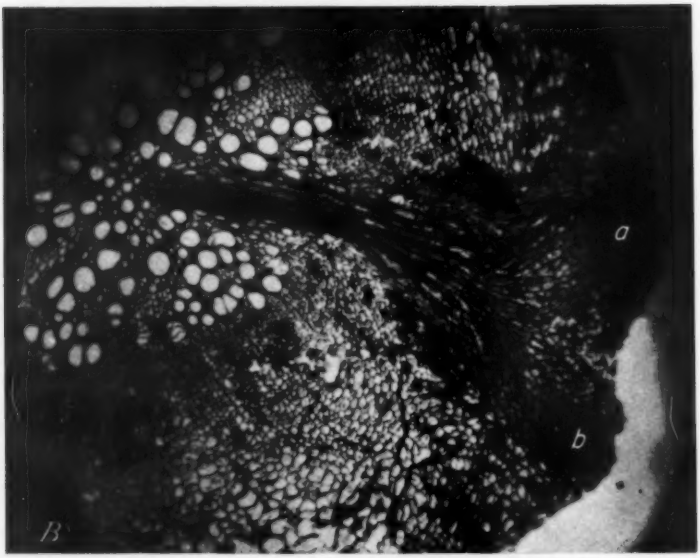


A, Transverse section through outer part of enlarged hypocotyl. Note active pericycle (*per*) and phloem (*ph*) and also the oil ducts (*od*). × 200. *B*, Cross section of a secondary root. Note numerous phloem fibers and lack of thin-walled parenchyma in both xylem and phloem: *ph*, Phloem; *ph fib*, phloem fibers; *per*, pericycle. × 200.





Origin of lateral roots during secondary growth from the primary root: *A*, Lateral root (*a*) originating from pericycle near trace formed by endogenous one, cortex still present. *B*, Origin of lateral roots (*a* and *b*) from older primary root after cortex is shed. × 103.

PRENATAL AND POSTNATAL SEX RATIO IN SHEEP [1]

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INTRODUCTION

Modern evidence indicates that the sex chromosome from the male gamete is the initial determiner of sex in mammals. A necessary implication of this mechanism is that sex of a zygote is determined at fertilization. Cytological studies indicate that the male gametes bearing, respectively, the X and the Y chromosome are produced in equal numbers. Unless factors other than chance interfere, the sex ratio at fertilization should accordingly be one of equality. In the absence of sex reversal, later change can come about in the ratio between the sexes only as the result of a differential mortality.

Sex ratios (percentages of males) may be ascertained at different stages in the life history of a population. In mammals, it is convenient to consider three separate stages: at conception, birth, and maturity. Sex ratios at these stages are usually spoken of as primary, secondary, and tertiary, respectively.

The true primary sex ratio might conceivably be obtained by a cytological study of a large number of fertilized eggs. The ratio would depend upon the proportion of ova fertilized by the male- and female-determining spermatozoa, respectively. However, adequate numbers of fertilized mammalian eggs for such a study, in which the cytological picture could be determined, would be difficult to obtain. It is also impracticable to make sex determinations on a sufficiently large number of embryos before external differentiation of the genitalia. The immense labor involved precludes the histological examination of the gonads of any large number of embryos as a laboratory method. Actually, therefore, primary sex ratios have not been determined, but an approach is made by a study of the percentage of males in fetal life. This method of approach, then, will give the sex ratio as early in prenatal life as the sexes can be determined macroscopically. It leaves the very early stages as a matter of speculation.

Sex ratios at birth in most mammals have been found to approach equality. In contrast to this, however, several studies of fetal sex ratios have indicated a preponderance of males before birth. In some cases this percentage has been reported as being high in early fetal stages and showing a gradual decline toward birth. Such a trend would indicate a higher proportion of males at conception than at any other time in the life cycle. Most authors have assumed that with a high primary sex ratio, differential prenatal mortality tends to

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[2] The author wishes to acknowledge his indebtedness to Drs. L. J. Cole and L. E. Casida, Department of Genetics, University of Wisconsin, for their guidance and encouragement, kind assistance, and patient and helpful criticism; to Col. E. N. Wentworth of Armour & Co., for his interest in securing the cooperation of the large Chicago packers in obtaining sheep fetuses for this study; and to Armour & Co. and Wilson & Co., as well as to a large group of his coworkers and associates, appreciation for their cooperation in making possible this study.

equalize the sexes by the time of birth. The only adequate data which give no evidence of difference between the prenatal and secondary sex ratios are those of MacDowell and Lord (*17*)[3] on the sex ratio of litters in the mouse.

The tertiary sex ratio is commonly applied to animals in the wild state where there are few artificial factors causing a differential mortality from birth to maturity. The tertiary sex ratio in domestic animals has little biological meaning because of the polygamous use of breeding males and the elimination of many males before they reach maturity.

The present paper reports the results of a study made on the prenatal and postnatal sex ratio in sheep.

REVIEW OF LITERATURE

General reviews of the literature on sex ratios have been presented by Parkes (*27*) and more recently by Johansson (*13*). Several authors have reported the secondary sex ratios in sheep. Their data have been obtained from flock book records, breeders' replies, and lambing records. A summary of the available secondary sex ratios reported in sheep (table 1) gives a male percentage of 48.96 ± 0.14 on a total of 127,587 individuals. This is slightly lower than 49.5 percent, reported by Lush (*16*). The fact that breeders' replies for some of the earlier work reported gave the ratio at castration time, and hence not strictly secondary ratios, may explain the slightly lower value for those records. McPhee (*21*) has shown for swine that herdbook data do not possess the necessary accuracy for a study of sex ratios and frequency of sex combinations.

TABLE 1.—*Sex ratios reported in sheep by various investigators*

Breed	Total amounts	Males	Authority	Source of data
	Number	*Percent*		
Leicester	8,965	44.07±0.48	Darwin (*9*)	Breeders' records—castration ratio.
Cheviot and Blackface.	50,685	49.40± .22	do	Do.
Cross-bred and Hampshire.	6,751	49.34± .61	Wilckens (*31*)	Breeders' replies.
Several breeds	821	49.94±1.74	Carlyle and McConnell (*2*).	Lambing records—Wisconsin Station.
Mixed breeding	371	54.40±2.58	Popence (*29*)	Bell's records.
Do	2,623	49.50± .98	Nichols (*23*)	Breeders' replies.
Do	17,201	49.34± .38	do	Breeders' replies—castration ratio.
Southdown	5,014	51.02± .71	Nichols, (*24*)	Private records.
Blackface	9,549	48.27± .51	Nichols, (*25*)	Breeders' records—castration ratio.
Cheviot	10,512	48.99± .49	do	Infantile ratio.
Several breeds	13,241	49.30± .43	Johansson (*14*)	Private records.
Grade Hampshire	1,019	48.40±1.57	Chapman and Lush (*4*).	Lambing records—Iowa Station.
Southdown	835	55.20±1.72	Phillips personal communication.	Lambing records—U. S. Department of Agriculture, Beltsville, Md.
Total	127,587	48.96±0.14		

Several authors have reported on the prenatal sex ratio of other mammals. The only direct evidence that the ratio at conception is not higher than that at birth is the investigation by MacDowell and

[3] Italic numbers in parentheses refer to Literature Cited, p. 578.

Lord (*17*, *18*, *19*), previously mentioned. They found that the sex ratio of 68 litters of mice, which was determined by corpora lutea counts, suffered no differential prenatal mortality. Of the 523 young in these litters, 261 were male, and 262 female (49.9 percent males), a ratio indistinguishable from the normal ratio at birth.

Information on sex ratio at birth and sex incidence of fetal mortality has been used to calculate a probable figure for the ratio at conception in man. Parkes (*28*) presented such material in a graph showing the decline in the sex ratio of mortality for the determinable part of the period of gestation as nearly linear. He then extrapolated the curve to the time of conception, thus arriving at a presumable sex ratio of mortality during the first 3 months of gestation, in which sex cannot be observed. He concluded that the primary sex ratio is probably about 120 males to 100 females (54.54 percent males).

Lillie (*15*), in his work on the freemartin, found that the fetal sex ratio for dizygotic twins in cattle was 134, which is much higher than the secondary sex ratio, 106, given by Johansson (*13*) for all births. Lillie did not classify the fetuses in age groups to ascertain if there was a trend in the mortality for the different stages of development. Jewell (*12*) found that the sex ratio of 1,000 cattle fetuses was 123.21 to 100, but observed no definite relation to stage of development.[4]

Parkes (*26*) found that the percentage of males was 56.8 ± 1.38 among 583 pig fetuses of all ages. This is higher than that of 51.14 ± 0.18 for pigs at birth, summarized from various authors by Lush (*16*). In addition, when these pig fetuses were classified by weight (0–100, 101–300 g, 301+g) the percentages for males were 59.1, 57.0, and 53.2, respectively. The percentage of males decreases as gestation proceeds, and Parkes concludes that this result can only be brought about by a differential mortality of males and females. Extrapolation of the foregoing data shows the primary sex ratio to be nearly 60 percent. Crew (*7*) made a similar investigation on the fetal pig and also concluded that the primary sex ratio must be at least 60 percent.

It appears that the only data recorded on sex of prenatal sheep are those of Malan and Curson (*22*), who report seven male and two female fetuses for the South African Merino.

MATERIAL, METHODS, AND RESULTS

THE SECONDARY SEX RATIO

Lambing Records of the Pennsylvania Experiment Station

The source of data for the secondary sex ratio is the lambing record of the registered flocks maintained at the Pennsylvania Agricultural Experiment Station from 1923 to 1937, inclusive. These lambing records include still births as well as lambs alive at birth and therefore are more complete than flock-book records which may be considered as the record of only the animals registered.

The mean percentage of males of 1,742 births for all breeds, including Southdown, over a period of 15 years is 50.2 ± 1.19 (table 2). For the same period, 433 births of registered Southdowns give a per-

[4] At the recent meeting (Nov. 1938) of the American Society of Animal Production, Chapman, Cassida, and Côté presented a paper on "Sex ratios of fetal calves." In this paper the authors presented evidence of a high sex ratio (66 percent males) in the earliest (5 to 10 cm fetal length) stage in which sex is macroscopically distinguishable.

centage of males of 50.3±2.40, which does not differ significantly from that for all breeds (table 2).

The data were examined to determine whether there is a significant difference in the percentage of males from year to year.

TABLE 2.—*Secondary sex ratio in sheep, 1923–37*

Year	All breeds including Southdown				Southdown			
	Males	Females	Total	Males	Males	Females	Total	Males
	Number	*Number*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Percent*
1923	17	22	39	43.6±7.94	0	4	4	
1924	66	43	109	60.6±4.68	10	7	17	58.8±11.9
1925	46	37	83	55.4±5.46	8	6	14	57.1±13.2
1926	49	31	80	61.3±5.45	13	4	17	76.5±10.3
1927	49	46	95	51.6±5.13	16	14	30	53.3± 9.11
1928	52	65	117	44.4±4.59	8	14	22	36.4±10.2
1929	69	61	130	53.1±4.38	11	18	29	37.9± 9.01
1930	45	59	104	43.3±4.86	13	14	27	48.1± 9.62
1931	67	73	140	47.9±4.22	20	16	36	55.6± 8.28
1932	61	69	130	46.9±4.38	13	18	31	41.9± 8.86
1933	58	69	127	45.7±4.42	20	24	44	45.5± 7.51
1934	57	54	111	51.4±4.74	24	16	40	60.0± 7.75
1935	63	58	121	52.1±4.54	21	15	36	58.3± 8.22
1936	90	95	185	48.6±3.67	21	25	46	45.7± 7.34
1937	86	85	171	50.3±3.82	20	20	40	50.0± 7.91
Total	875	867	1,742	50.2±1.20	218	215	433	50.3±2.40

The extremes of variation in sex ratio are 43.3±4.86 in 1930 and 61.3±5.45 in 1926, with a mean difference of 18±4.92 percent. A chance difference of such magnitude is not unlikely when selected in this manner from a large number of differences. A χ^2 test of these 14 yearly percentages shows them not to be heterogeneous.

THE SOUTHDOWN FLOCK-BOOK RECORDS

A true tertiary sex ratio in domestic animals is difficult to obtain because of the elimination of males for economic reasons. An approach to it is made, however, by studies of herdbook records.

Flock-book data on sheep registered as association numbers 30,000 to 35,000 (16–17, American Southdown Record) were used as representing a typical cross section of registrations in this breed. Practically all of these animals are entered in the flock book before they are 1 year of age. Registrations of the lifetime progeny records of 1,536 ewes furnished the material for this study. Records were assembled in which the following information was available for each birth represented by registered progeny from these ewes: (1) Number of progeny at the birth; (2) sex of at least one registered offspring from the birth; (3) size of litter in which the dam of each birth was born, that is, whether the dam herself was dropped as a single or one of a multiple birth. The number of births for which the records yielded the desired information were 6,116 for (1), 6,065 for (2), and 6,050 for (3).

Single births constitute 55.73 percent of the lambings registered from these ewes; twin births 43.42 percent; and the remainder, less than 1 percent, triplets and quadruplets. The proportion of males is 32.44±0.80 percent for singles and 37.49±0.71 for twins (table 3).

Combined, these give 35.35 percent of males, which is the sex ratio of registration (disregarding the inconsiderable number of triplets and quadruplets).[5] The low percentage of males is accounted for by the fact that only a portion of the ram lambs are reserved and registered. For this reason, flock-book records have little value for a study of natural sex ratios and twin combinations.

TABLE 3.—*Sex ratio of registration with twin combinations as shown by the Southdown flock-book records*

Type of birth	Births	Combinations	Lambs registered	Males registered
	Number		*Number*	*Percent*
Single	1,106	1 ram	1,106	32.44±0.80
	2,303	1 ewe	2,303	
Twin	339	2 rams	678	37.49±.71
	808	1 ram, 1 ewe	1,616	
	847	2 ewes	1,694	
	257	1 ram, 1 unregistered	257	
	405	1 ewe, 1 unregistered	405	
Total	[1] 6,116		8,059	

[1] Total does not include 48 sets of triplets and 3 quadruplets.

The difference in percent of males as between single births and twins suggests that there may exist a slight preference for selecting rams that are born as one of a pair of twins.

Records secured for twin combinations in which both members of the pair were registered gave 339 ♂ ♂, 808 ♂ ♀, and 847 ♀ ♀ (table 3). The wide departure from the 1 : 2 : 1 ratio is obviously attributable to the excess of females over males registered.

Although not directly pertinent to this study, it is interesting to compare the type of birth of the offspring with that of the dam (table 4).

Of 4,309 lambings by ewes which were themselves born as singles, 58.18 percent were singles and 41.82 percent twins. In 1,741 cases in which the ewe herself was a twin, 51.23 percent of the lambings were singles and 48.77 percent twins. These data show a significant tendency for ewes themselves born as one of a pair of twins to produce more twins than do ewes born as singles. Heredity is undoubtedly a factor in producing this result, but similarity of flock conditions from one year to another may also play a part.

TABLE 4.—*Inheritance of tendency to multiple births in sheep*

Dams (number)	Type of birth	Offspring			
		Singles		Twins	
		Number	*Percent*	*Number*	*Percent*
4,309	Single	2,507	58.18±0.98	1,802	41.82±1.16
1,741	Multiple	892	51.23±1.67	849	48.77±1.71

[5] For convenience the term "twins" is used for multiple births in general and may include an insignificant number of triplets or quadruplets unless otherwise qualified.

Fetuses were secured from ewes for which the time of the breeding that produced conception was known and hence the age of the fetus could be determined. An additional number of preserved fetuses was secured, and approximately 700 gravid uteri from freshly killed dams were examined in Chicago packing plants.

Timed Fetuses

During the breeding season of 1934, 100 old Merino ewes were secured. They were divided into two lots and mated with two

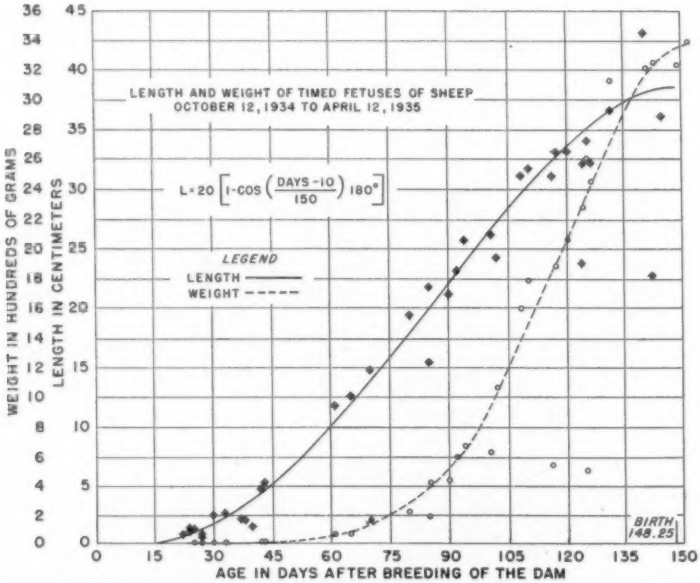

Figure 1.—Length and weight of fetus as related to its age in days after dam was bred

registered Shropshire rams. Records of mating were made each day by a college-trained assistant. Ewes were then sacrificed at intervals during pregnancy to secure as complete a series as possible of fetuses of definitely known ages up to and including full term. Thirty-eight such fetuses were obtained, and four ewes were permitted to lamb at normal term. The periods of gestation of these four were 147, 147, 148, and 151 days.

The fetus from ewe No. 47 was located in the vagina, partly resorbed but in the process of abortion. The fetuses from ewes Nos. 18, 22, and 29 were secured after the dams had died from natural causes, and this may explain the slight irregularities in the records of these fetuses. The data secured on the timed fetuses are presented in table 5. In figure 1, the two curves show the general relationship of

length and weight of fetus to age in days, age being determined from date of breeding the dam. With the length of any sheep fetus known, the approximate age in days may be calculated by the following formula: [6]

$$\text{Age in days} = 10 + \tfrac{5}{4} \text{ arc cos} \left(1 - \frac{L}{20}\right)$$

(where arc cos is measured in degrees and L=length in centimeters).

The curves are very similar to those of Curson and Malan (*8*), and also those of Winters and Feuffel (*32*).

TABLE 5.—*Length and weight of sheep fetuses as related to their age and sex from work at the Pennsylvania Agricultural Experiment Station* [1]

Ewe No.	Weight of ewe	Age of fetus	Length of fetus	Weight of fetus	Sex of fetus	Ewe No.	Weight of ewe	Age of fetus	Length of fetus	Weight of fetus	Sex of fetus
	Pounds	*Days*	*Centimeters*	*Grams*			*Pounds*	*Days*	*Centimeters*	*Grams*	
82	85.5	21	0.89			43	77.0	92	23.00	595.3	Female.
94	77.0	24	1.28			25	67.0	94	25.50	652.0	Male.
68	70.0	24	1.29			66	74.0	101	26.00	620.0	Female.
54	91.0	24	1.38	1.4		75	68.0	102	24.00	1,047.2	Male.
22 [2]	85.0	25	1.14	1.5		21	82.0	108	31.00	1,587.6	Do.
86	81.0	27	.56			28	93.0	110	31.50	1,673.8	Do.
65	88.0	27	.70			29 [2]	92.0	116	31.00	539.0	Do.
58	72.0	30	2.39	5.6		10	91.0	117	33.00	1,872.7	Do.
11	86.0	33	2.68	1.6		64	92.0	120	33.00	2,040.6	Female.
77	76.0	37	2.09			16	79.0	124	32.00	2,270.0	Do.
8	98.0	38	1.97			31	83.5	125	34.00	2,598.2	Male.
47 [2]	74.0	40	1.35			39	72.0	125	23.50	482.0	Female.
67	91.0	42	4.56	7.0		20	92.0	126	32.00	2,440.1	Male.
85	100.0	43	5.12	8.6	Male.	12	81.0	131	36.50	3,120.9	Female
51	82.0	61	11.60	56.9	Do.	95	79.0	140	43.00	3,206.3	Do.
18 [2]	82.0	65	12.50	60.4	Do.	81	85.5	142	22.50	3,234.6	Male.
36	73.0	70	14.70	155.9	Female.	92	90.0	144	36.00	4,876.2	Do.
72	81.0	80	19.30	210.0	Do.	6 [2]	113.0	147		4,082.4	Do
97	97.0	85	15.30	170.1	Do.	7 [2]	106.5	151		3,401.9	Female
88	81.0	85	21.50	411.0	[3]Male.	9 [2]	95.0	148		3,220.6	Male.
33	80.0	90	21.00	429.3	Do.	91 [2]	98.0	147		3,628.7	Do.

[1] Data collected between Oct. 12, 1934, and Apr. 12, 1935.
[2] Ewes died or otherwise excluded.
[3] Data at parturition.

PACKING-HOUSE MATERIAL

Arrangements were made through the courtesy of Armour & Co. and Wilson & Co. to obtain sheep fetuses at their packing plants in Chicago. At first the entire uteri were placed in preservative fluid and shipped to the Department of Zoology, Pennsylvania State College, where they were measured and weighed, and the sex was recorded. Later, in order to secure larger numbers and more complete data, the work was done directly at the packing plants, the fetuses being obtained directly on the killing floors and records taken on the day of slaughter or the day following. Length was taken as the crown-rump measurement in a straight line from the cephalic curvature to caudal extremity (*1*) in all cases, with the exception of the very smallest specimens, in which the neck-rump length was secured.

All uteri containing either dead or live fetuses were used in this study. The uteri were removed by severing the vagina posterior to

[6] Credit is gratefully given to Prof. H. N. Benkert, Department of Civil Engineering, and Dr. Evan Johnson, Jr., Department of Mathematics, Pennsylvania State College, for assistance with the formula and growth curves.

the cervix. At the time of collection of this material (March 3–14, 1936), many pregnant fat ewe lambs came to the market.

Records of sex and length of fetus, together with the position and number of corpora lutea, are used in the study of the fresh material. Corpora lutea counts were not available in the preserved material.

The percentage of males for the preserved fetuses of 1933 was 43.9 ± 7.75; for 1934 it was 41.9 ± 5.74, and for 1935 it was 52.2 ± 3.96. These year-to-year differences, however, are not statistically significant.

The 25 timed fetuses of 1935 classified for sex gave a percentage of males of 60.0 ± 9.80. The percentage of males was 51.7 ± 1.93 for 673 fetuses examined from fresh packing-house material in 1936. When all the fetuses are grouped together, there is a total of 972 (495 males, 477 females), giving a percentage of males of 50.9 ± 1.60 (table 6). This is essentially the 1:1 ratio which would be expected if the male- and female-producing gametes are themselves produced in equal numbers, if chance alone is operating in fertilization, and if there is no sex reversal or differential mortality during intra-uterine life.

The mean percentage of males of 1,742 births for all breeds over a period of 15 years at the Pennsylvania Station is 50.2 ± 1.20. This secondary sex ratio is essentially the same as the above prenatal sex ratio of $50.9 \pm .60$.

The fetuses were grouped in classes according to length, 5-cm differences being used as a basis of classification (table 7). These differences in length naturally correspond roughly with differences in age (fig. 1). No consistent trend in the percentage of males is apparent, and the χ^2 test shows that the variation is not significant. The same is true when they are grouped in 10- and 20-cm classes. The first group in the 5-cm classification, with a sex ratio of 80.0 ± 6.76, is significantly different from the others, but the determination of the sex on some of these very early embryos is questionable. There is no indication, however, of differential mortality of the sexes after the 5-cm stage.

TABLE 6.—*Summary of data on prenatal sex ratio in sheep*

Year	Fetuses used	Singles		Twins			Triplets	Total fetuses	Males
		Male	Female	Male-male	Male-female	Female-female			
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1933	Preserved material	16	19	0	2	1	0	41	43.9±7.75
1934	do	25	31	1	4	4	0	74	41.9±5.74
1935	do	81	70	0	2	2	0	159	52.2±3.96
1935	Timed fetuses	15	10	0	0	0	0	25	60.0±9.80
1936	Fresh material	254	237	25	41	22	[1] 2	673	51.7±1.93
	Total	391	367	26	49	29	2	972	50.9±1.60

[1] In 1 litter, 2 males, 1 female; in the other, 1 male, 2 females.

The prenatal sex ratio has been considered in relation to the ovary from which the ova originated (table 8). There were 232 fetuses attributed to single ovulations from the right ovary as indicated by the location of the corpora lutea. The percentage of males for these

was 53.0±3.27. A total of 185 fetuses originating from ova shed singly by the left ovary gave a percentage of males of 49.2±3.67. The two percentages are not significantly different. Similarly, there was no significant difference in the percentage of males of fetuses attributable to either the right or the left ovary when there were two corpora in one and none in the other, or when there was one corpus luteum in each. The number of cases in which there were two corpora in one ovary and one in the other was so small that conclusions on this distribution are unwarranted. The percentage of males, however, for these few was 50.00.

TABLE 7.—*Prenatal sex ratio according to length of fetus*

Length of fetus (centimeters)	Males	Females	Total fetuses	Males	Length of fetus (centimeters)	Males	Females	Total fetuses	Males
	Number	*Number*	*Number*	*Percent*		*Number*	*Number*	*Number*	*Percent*
Under 5.0	28	7	35	80.0±6.76	30.0–34.9	19	18	37	51.4± 8.22
5.0–9.9	81	80	161	50.3±3.94	35.0–39.9	18	7	25	72.0± 8.98
10.0–14.9	133	164	297	44.8±2.89	40.0–44.9	10	9	19	52.6±11.40
15.0–19.9	115	90	205	56.1±3.46	45.0 and over	3	1	4	75.0±21.70
20.0–24.9	56	65	121	46.3±4.53					
25.0–29.9	32	36	68	47.1±6.05	Total	495	477	972	50.9±1.60

In general, no significant differences in sex ratio could be attributed to the total number of ova ovulated or to the ovary from which the ova originated. The percentage of males was 51.3±2.45 when one corpus luteum was present, 53.92±3.39 when two were present, and 50.00±14.42 when three were present. When all of the ova came from the right ovary, the percentage was 54.3±2.82; when from the left ovary, it was 49.8±3.32; and when from both ovaries at the same time, it was 50.9±4.86.

TABLE 8.—*Sex ratio of living sheep fetuses produced by right and left ovaries*

Corpora lutea in—		Fetuses appearing as—							Total fetuses	Males
		Singles		Twins			Triplets			
Right ovary	Left ovary	Male	Female	Male-male	Male-female	Female-female	Male-male-female	Male-female-female		
Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1	0	121	109	2					232	53.0± 3.27
0	1	91	94						185	49.2± 3.67
2	0	10	7	22	30	12			81	58.0± 5.49
1	1	16	14	16	32	16			94	51.1± 5.16
0	2	7	3	8	14	10			42	52.4± 7.71
2	1				2				2	50.0
1	2			2		2	3	3	10	50.0
All fetuses									646	52.2± 1.96
1 corpus luteum present									417	51.3± 2.45
2 corpora lutea present									217	53.9± 3.39
3 corpora lutea present									12	50.0±14.42
Corpora lutea in right ovary only									313	54.3± 2.82
Corpora lutea in left ovary only									227	49.8± 3.32
Corpora lutea in the right and the left ovary									106	50.9± 4.86

In the ovaries studied, a total of 413 corpora lutea (58.58±1.85 percent) were found in the right ovary and 292 in the left ovary. In this respect the sheep resembles other domesticated animals that have been investigated. Parkes (*26*) and Crew (*7*) found an excess of ovulations occurring in the right ovary in the pig; C. F. Clark (*5*), Casida and Venzke (*3*), and Reece and Turner (*30*) found this true in cattle; and since the present study was completed McKenzie and Terrill (*20*) have reported 56.23 percent of the ovulations in the right ovary in the ewe.

An estimate was made of the incidence of fetal mortality, based on the discrepancy between the number of corpora lutea present and the number of live fetuses in the uteri (table 9). Of necessity only those cases were studied in which there was persistent evidence of a pregnancy, that is, live or dead fetuses or remnants of fetal membranes. Consequently, cases of absence of fertilization or early embryonic death with complete resorption could not be included. In cases of multiple ovulations in which there were discrepancies between the number of corpora lutea and total number of fetuses, it is not known whether there had been additional fetuses resorbed early or the additional ova had not been fertilized. In general, therefore, the estimate of mortality (embryonic and fetal death, and failure in fertilization of eggs) must be considered as a minimum, especially in the cases of single ovulations.

Although 772 corpora lutea were observed in the ovaries associated with pregnant uteri, 16.04±1.32 percent of these were not accounted for by the presence of live fetuses. The percentage of dead or missing fetuses was 8.15±1.28 with one corpus luteum present, 26.17±2.55 with two corpora lutea, and 42.86±10.80 percent with three.

TABLE 9.—*Mortality of sheep fetuses in relation to number of corpora lutea*

Corpora lutea (number)	Fetuses found in uteri	Live	Dead or missing	Total live, dead, or missing	Dead or missing
		Number	*Number*	*Number*	*Percent*
1	1 live	415			
1	1 dead		37		
1	2 live	2			
Summary		417	37	454	8.15±1.28
2	2 live	160			
2	1 live, 1 dead or missing	60	60		
2	2 dead or missing		18		
Summary		220	78	298	26.17±2.55
3	3 live	6			
3	2 live, 1 dead or missing	6	3		
3	3 dead or missing		6		
Summary		12	9	21	42.86±10.80
Total		649	124	773	16.04±1.32

This result indicates a decidedly significant increase in the mortality of the fetuses (or possibly failure of fertilization) with increase in number of ova shed, as indicated by the number of corpora lutea. This conclusion agrees substantially with that of Hammond (*10*), who

found in his study of fetal atrophy in sheep that it increased rapidly with the number of fetuses in the uterus, and that in most cases death of the fetus occurred during the early stages of development. Winters and Feuffel (*32*) state that nearly 8 percent of the eggs ovulated did not develop.

If there is a differential mortality with respect to sex during the intra-uterine period it should be reflected as a difference in sex ratios between dead and living fetuses. A summary of the sex ratio in relation to mortality is presented in table 10. The data are analyzed with reference to number of corpora lutea and number of live or dead fetuses. While the actual ratios obtained differ considerably, in no cases are their differences significant. There is, therefore, no evidence in this study for a differential intra-uterine mortality with respect to sex. This is further substantiated if only those cases are taken in which the number of corpora lutea corresponds exactly with the number of live fetuses found, and which, if the evidence is accepted, indicate no previous prenatal mortality whatever. The ratio for 575 such fetuses is 51.5±2.08. These data are similar to those of MacDowell and Lord (*17, 18*) except that all of their sex records were at birth while these are at various stages and consequently do not exclude the possibility that there might have been some later mortality if gestation had not been terminated.

TABLE 10.—*Sex ratio in relation to sheep-fetus mortality*

Corpora lutea (number)	Fetuses found in uteri	Fetuses	Males	
		Number	*Number*	*Percent*
1	1 live	415	212	51.1± 2.45
1	1 dead	16	7	43.8±12.40
2	2 live	160	84	52.5± 3.95
2	1 live	57	33	57.9± 6.54
2	1 or 2 dead	6	4	66.7±19.20
Live fetuses where—				
Number of corpora lutea equal number of live fetuses.		575	296	51.5± 2.08
Number of corpora lutea is greater than number of live fetuses.		57	33	57.9± 6.54
Dead fetuses, sex determinable		22	11	50.0±10.70

MULTIPLE BIRTHS—FREQUENCY OF SEX COMBINATIONS

A study of the sex combinations in twins may be used to estimate the frequency of monozygotic twinning. In material in which no monozygotic twinning occurs and in which the sex ratio is equal, the ratios of the three possible sex combinations would be expected to approximate 1♂♂ : 2♂♀ : 1♀♀, within the limits of error of random sampling and with the provision that these ratios are not materially changed by selective prenatal mortality. Monogyzotic twinning should raise the proportion of like-sexed twins. Only limited data are available on the frequency of the different sex combinations in multiple births in sheep. Both R. T. Clark (*6*) and Johansson (*14*), who have investigated the problem, conclude that monozygotic twinning is at most a comparatively rare phenomenon in sheep.

Totals of lambing records at the Pennsylvania Station over a period of 15 years, excluding the Southdowns, gave 112♂♂ : 214♂♀ :

95 ♀ ♀, twin combinations (table 11). For the same period the Southdowns yielded sets of twins in the following ratios: 23 ♂ ♂ : 60 ♂ ♀ : 39 ♀ ♀. The χ^2 test shows the variation from the expected to be insignificant in both cases and also for the totals. The combinations of twins for all prenatal records are 26 ♂ ♂ : 49 ♂ ♀ : and 29 ♀ ♀; again not a significant departure from the 1:2:1 ratio.

TABLE 11.—*Twin combinations in sheep*

[From lambing records at the Pennsylvania Agricultural Experiment Station]

Item	Male-male	Male-female	Female-female
	Number	*Number*	*Number*
All breeds (excluding Southdown)	112	214	95
Southdown only	23	60	39
Total lambing records	135	274	134
Total prenatal records	26	49	29
Total all-twin records	161	323	163
	Percent	*Percent*	*Percent*
Ratio all-twin records	24.9	49.9	25.2

Grouping all the data together gives sex-combination ratios of 161 ♂ ♂ : 323 ♂ ♀ : 163 ♀ ♀. This total is essentially a 1 : 2 : 1 ratio, and accordingly there is no evidence from the twin combinations that there is any differential mortality of the sexes either during intrauterine life or at the time of birth.

The absence of an excess of like-sexed twins indicates that, at most, monozygotic twinning in sheep is rare. The one case of twins with only one corpus luteum present (table 9) which has already been described Henning (*11*) makes it seem highly probable, however, that monozygotic twinning may occur.

DISCUSSION

The normal secondary sex ratio (proportion of males at birth) in mammals appears in most cases for which adequate data are available to be remarkably close to 50 percent. In those species for which it is best established it varies less than 2 percent from equality. (See Lush (*16, p. 315*). The mule is not a species, and the ratio for the cat is based on embryos.) This would appear to be the natural consequence of a system of alternative sex determination based on equal numbers of unlike male gametes, providing fertilization is a random matter and differential mortality of the sexes from fertilization to parturition is slight. Under such an interpretation it might be assumed that the deviation from equality at any time during fetal life would be less than at birth. If the deviation were greater in one direction at any particular stage, because of a higher mortality of one sex, it would have to be compensated for by an increased mortality of the other sex at another stage to result in the approximate equality at birth. This would presuppose a rather nice balance of the mortality rates at different periods. The simpler expectation would be a steadily increasing deviation, except for random divergences, from a start at equality to the particular ratio characteristic for the species at birth.

Critical data on mammalian prenatal sex ratios, based on actual determinations of the sex of large numbers of fetuses of known age, are relatively few. This has been supplemented, in some instances, by records of prenatal mortality, which can be used to estimate indirectly the prenatal sex ratio. For the most part, particularly in man, the pig, and the cow, these records indicate a situation quite different from the simple straight-line divergence from initial equality suggested above. In general, in these species there appears to be a considerable excess of males during intra-uterine life and both Crew (*7*) and Parkes (*26*) have argued that this predicates an even higher primary male sex ratio. Parkes has stated this argument clearly for the pig. He concludes (*26, p. 298*):

> * * * the percentage of males decreases as gestation proceeds, a result which can only be brought about by a differential mortality of males and females. It would appear that the percentage at conception must be very near 60 per cent or 150 males per 100 females.

The initial ratio for man has been assumed to be about the same.

The careful observations of MacDowell and Lord (*17, 19*) on the mouse give results quite contrary to the conclusions drawn from previous data. These authors showed that when there was no embryonic mortality, as indicated by a correspondence between the number of corpora lutea of pregnancy and the number of young born, a 1 : 1 sex ratio resulted (*17*). Furthermore, when prenatal mortality has occurred, as shown by a discrepancy between the number of corpora lutea and the number of young in the litter, the amount of such mortality is not correlated with the sex ratio (*19*). From the first observation it is concluded that in mice "the primary sex ratio is * * * almost exactly 1 : 1," and from the second "that during gestation in mice there is no continuous selective elimination of one sex or the other before birth."

The conclusions drawn from the data on sheep, presented in this paper, appear to agree more closely with those for the mouse. It is true that the sex ratio for embryos of the smallest class (under 5 cm) is high and significantly so, but there is reason to doubt the accuracy of some of the determinations in this class. From these smallest embryos to birth the deviations are random and insignificant; certainly they show no definite trend.

How may these discordant results and conclusions for different species be explained? There are several possible suggestions:

(1) That there are species differences with respect to the primary sex ratio.

(2) That the primary sex ratio is essentially one of equality, but that in some species a high mortality of females in the early stages, before sex is ordinarily distinguishable, is followed by a later compensating mortality of males. In assuming that the trend in the later stages of pregnancy may be projected to estimate the primary sex ratio, little consideration has been given to the factors that might be causing a differential mortality during this earlier period. In the sheep, for example, distinguishable external differentiation of sex does not occur until about 45 days of pregnancy, which is about one-third of the whole period.

(3) That the data are in most cases too few or not sufficiently critical to permit definite and final conclusions.

The last suggestion emphasizes the need of more critical data, not only on the sheep, but on as many mammalian species as possible.

SUMMARY

An examination of the progeny records of the ewes among 5,000 sheep registered in the American Southdown Record gave a total of 6,116 registered Southdown births—3,409 singles, 2,656 twins, 48 triplets, and 3 quadruplets.

The frequencies of sex combinations in 1,994 complete twin-progeny records (that is, with both twins registered) gave 339 ♂ ♂ : 808 ♂ ♀ : and 847 ♀ ♀.

Of the animals registered as singles or twins in the American Southdown Record, 35.35 percent are males. This deficiency of males results from the commercial practice of reserving more females than males for breeding purposes.

A summary of 1,742 lambing records of all breeds kept at the Pennsylvania Agricultural Experiment Station gave 875 males and 867 females, with a sex ratio of 50.20 ± 1.20. Another summary of 433 lambing records of the Pennsylvania Station on registered Southdowns gave 218 males and 215 females, with a sex ratio of 50.30 ± 2.40.

The secondary sex ratio from these combined records is 50.20 ± 1.20.

A total of 972 prenatal lambs (495 males and 477 females) gave a sex ratio of 50.9 ± 1.60.

Prenatal growth curves are presented for length and weight.

A formula is given for estimating the fetal age of sheep from length in centimeters.

Mortality of sheep fetuses increases directly as the number of corpora lutea increases from 1 to 3.

There is no statistically significant difference between the sex ratios of the live and dead fetuses, or of fetuses deriving from ova from the right or left ovary, or both.

There is no evidence of a significantly higher mortality of either sex during the intra-uterine period.

Ovulation in the ewe is appreciably more frequent in the right ovary (58.58 ± 1.85 percent) than in the left.

The prenatal sex ratio found in sheep is essentially one of equality (50.9 ± 1.60).

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SCHIZONOTUS SIEBOLDI, AN IMPORTANT PARASITE OF THE IMPORTED WILLOW LEAF BEETLE (PLAGIODERA VERSICOLORA) [1]

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INTRODUCTION

Schizonotus sieboldi (Ratz.) is a chalcidoid parasite of the imported willow leaf beetle (*Plagiodera versicolora* (Laich.)) and some other closely allied chrysomelids. *Plagiodera* was first recorded in the United States by Schaeffer (*9*) [3] in 1915, but it was undoubtedly well established at that time, for in 1916 Weiss (*13*) noted its presence in three localities in New Jersey. Since then it has become a serious pest of willows in the Northeastern States. Collections made in 1932 in the vicinity of Boston, Mass., included pupae heavily parasitized by *S. sieboldi*. A study has therefore been undertaken of the biology and effectiveness of this parasite.

REVIEW OF LITERATURE

Schizonotus sieboldi has been placed in the chalcidoid family Cleonymidae by Ashmead (*1*), but Ferrière and Faure (*3*) believe it should be considered a pteromalid. Apparently very little has been written regarding the biology of any of the species of Cleonymidae. Parker has not included a representative of this family in his work on chalcidoid larvae (*7*).

Although *Schizonotus sieboldi* has frequently been noted as a parasite of *Plagiodera* and allied Chrysomelidae, about the only information recorded on its biology is that given by Cushman (*2*) in 1917. He reared it on *Chrysomela interrupta* F. and briefly noted oviposition, development, and extent of parasitization, figuring the parasite larva and pupa in situ beneath a host pupa. In 1932 Jones (*4*) recorded the numbers of *Plagiodera* pupae parasitized in small collections made at Dracut and Milton, Mass.

Brief notes on another species of *Schizonotus*, *paillotti* Ferr. and Faure, have been made by the describers (*3*). This species is a hyperparasite of *Apanteles glomeratus* L. It feeds gregariously and overwinters as a larva.

DISTRIBUTION AND HOST RELATIONSHIPS

Schizonotus sieboldi is widely distributed in the United States and Europe. It was originally described from Germany, and has been

[1] Received for publication July 18, 1938. This study was conducted under the direction of C. W. Collins at Melrose Highlands, Mass., in 1934, and under the direction of R. C. Brown at Melrose Highlands, Mass., in 1935, and New Haven, Conn., in 1936.

[2] The writer is indebted to R. T. Webber and D. E. Parker for observations made in 1934, to H. A. Bess for making many of the pupal collections, and to Mary F. Benson for drawing the adult.

[3] Italic numbers in parentheses refer to Literature Cited, p. 592.

recorded from France and Italy. The Division of Insect Identification, Bureau of Entomology and Plant Quarantine, records it in this country from Virginia in 1881, Florida in 1903, Minnesota in 1914, Pennsylvania in 1916, and Massachusetts in 1932, and has suggested that, since it was a parasite of native species of chrysomelids before *Plagiodera* became established in the United States, it may be a circumpolar species.

Host records, also compiled in the Division of Insect Identification, list *Schizonotus* as a parasite of *Plagiodera versicolora*, *Chrysomela interrupta*, and *C. scripta* (F.) in the United States. In the European literature it is also recorded as a parasite of *C. populi* L. by Ratzeburg (*8*), and Scheidter (*10*) reared it from *C. vigintipunctata* Scop. During the course of this study it was reared as a parasite of *Plagiodera* from every point where host material was collected, from Washington, D. C., to Bar Harbor, Maine, and west to Little Falls, N. Y. No special attempt was made, however, to get the limits of *Plagiodera* distribution.

ECONOMIC IMPORTANCE

Plagiodera versicolora passes the winter as an adult beetle under loose pieces of bark or in crevices in the tree. In the vicinity of Boston, Mass., the adults become active about May 1, and they continue to emerge from hibernation during May. The first eggs are deposited about May 15, and the first pupae are formed about June 10. There are three generations and a partial fourth during the summer. Practically all the beetles go into hibernation during August. As the various generations overlap, *Plagiodera* in all stages of development may be found in the field from June until September, although at certain times one stage may predominate.

Schizonotus is a parasite of *Plagiodera* pupae. To check its importance as a control factor, collections of pupae were made weekly during 1934 and 1935 at three points near Boston, and in 1936 one collection was made at each of these points and several collections were made in the vicinity of New Haven, Conn. Care was taken to collect only fresh pupae, i. e., pupae that had not begun to darken. In general, such pupae were not over 2 or 3 days old. If they had been collected indiscriminately, a disproportionate number would have produced parasites, for parasitized pupae may be in the field 12 or 13 days, whereas adult beetles issue from unparasitized pupae in 3½ to 4 days. An attempt was made to have each collection include 100 pupae, and at least 2 hours was spent in searching whenever the full number was not obtained. The pupae were isolated immediately upon receipt at the laboratory, and were held for emergence of adults and parasites. It would have been desirable to make all the collections on infested willow trees, but this proved very difficult, since a comparatively small number of leaves could be examined from the ground and as the season progressed many of the leaves were so dry and brown that there was little feeding on them. Consequently the pupae were collected from leaves of underbrush upon which full-grown larvae had fallen and pupated.

Tables 1 and 2 show the extent of parasitization for the various collections.

TABLE 1.—*Parasitization of Plagiodera versicolora pupae by Schizonotus sieboldi in 1934, 1935, and 1936*

Place	Date of collection	Pupae collected	Pupae dead from unknown cause	*Plagiodera* adults obtained	Pupae parasitized by *Schizonotus*	
	1934	*Number*	*Number*	*Number*	*Number*	*Percent*
Milton, Mass.	July 12	100	6	12	82	82
	July 19	100	11	18	71	71
	July 25	100	5	28	67	67
	Aug. 2	28	6	5	17	60
	Aug. 9	58	5	0	53	91
	1935					
	June 21	100	12	59	29	29
	June 28	100	13	44	43	43
	July 5	24	3	19	2	8
	July 11	100	12	33	55	55
	July 19	100	8	39	53	53
	July 25	100	8	15	77	77
	Aug. 2	100	6	18	76	76
	Aug. 8	100	1	18	81	81
	Aug. 15	56	8	12	36	64
	1936					
	July 31	100	7	25	68	68
	1934					
Belmont, Mass.	July 17	100	4	51	45	45
	July 24	100	5	30	65	65
	July 31	100	24	9	67	67
	Aug. 7	11	2	1	8	72
	1935					
	June 17	100	9	61	30	30
	June 24	100	22	28	50	50
	July 2	100	5	48	47	47
	July 9	100	7	73	20	20
	July 17	100	4	26	70	70
	July 23	98	12	12	74	75
	July 30	100	9	14	77	77
	Aug. 6	100	10	18	72	72
	Aug. 12	100	13	31	56	56
	Aug. 19	100	12	14	74	74
	1936					
	July 31	100	11	8	81	81
	1934					
Saugus Mass.	July 13	100	2	32	66	66
	July 20	100	13	17	70	70
	July 27	100	7	63	30	30
	Aug. 3	100	10	32	58	58
	Aug. 10	20	2	9	9	45
	1935					
	June 20	96	22	34	40	42
	June 26	100	15	29	56	56
	July 3	97	17	38	42	43
	July 10	100	16	69	15	15
	July 17	100	10	17	73	73
	July 23	100	8	23	69	69
	Aug. 1	100	5	42	53	53
	Aug. 7	100	7	42	51	51
	Aug. 13	100	3	38	59	59
	Aug. 19	76	9	35	32	42
	1936					
West Haven, Conn.	July 31	100	19	14	67	67
Hamden, Conn.	Aug. 8	220	53	70	97	44
Foxon, Conn.	July 30	200	7	94	99	49
	Aug. 5	100	24	17	59	59

The data from Milton, Belmont, and Saugus show a rather high percentage of parasitization throughout the season in 1934 and 1935, and the one collection made in 1936 indicates that the same condition prevailed a third year. Collections made in the vicinity of New Haven

also showed high parasitization. In 1935 the collections made about July 10 at each point near Boston showed a decided drop in parasitization. A possible explanation of this is that the overwintering *Schizonotus* had died and only a few of the first-generation adults had

FIGURE 1.—*Schizonotus sieboldi.* Adult female.

issued and begun oviposition. The last of the female parasites that hibernated at the laboratory died on July 1.

TABLE 2.—*Average percentage of parasitization of Plagiodera versicolora by Schizonotus sieboldi in 1934 and 1935*

Place	Year	From all collections	From collections July 11 to Aug. 19 [1]
		Percent	*Percent*
Milton, Mass.	1934	73	73
	1935	58	68
Belmont, Mass.	1934	59	59
	1935	57	71
Saugus, Mass.	1934	55	55
	1935	50	60

[1] These figures may furnish a more accurate basis for comparison of the 2 years than those in the preceding column, for collections were not begun until July 12 in 1934, and parasitization becomes somewhat higher as the season progresses.

In spite of the high degree of parasitization, the intensity of the host infestation at Milton, Belmont, and Saugus remained severe for 3 years and appeared to be about the same each year. The trees at Milton and Belmont were entirely browned, practically every leaf being skeletonized, and at Saugus feeding was noticeable. The three collections in the vicinity of New Haven also showed a high degree of

parasitization. Feeding was severe at Hamden and West Haven, but hardly noticeable at Foxon. The percentages of parasitization were very nearly the same at all points, and the fact that feeding was less severe at Saugus and Foxon cannot be attributed to the work of the parasite unless the host populations at these points were considerably lower throughout the season. It will, of course, be necessary to devise a method of accurately measuring the host population before the true relation of this parasite to its host can be judged. Apparently, though, a severe infestation can persist for 3 years in spite of relatively high and fairly constant parasitization.

DESCRIPTIONS

ADULT

The adult (fig. 1) was originally described under the genus *Pteromalus* by Ratzeburg (*8, p. 230*) in 1852.

EGG

The egg (fig. 2, *B*) averages 0.42 mm long and 0.15 mm wide. It is cylindrical, rounded at the ends, and slightly widened at the cephalic extremity. The chorion is shiny white, with a slightly roughened area at the micropyle. One side is very finely reticulated. The sculpture is similar to that figured by Smith (*11*) for *Perilampus chrysopae* Crawf. and by Parker (*7*) for *P. tristis* Mayr, although in the eggs of those species the entire chorion is sculptured.

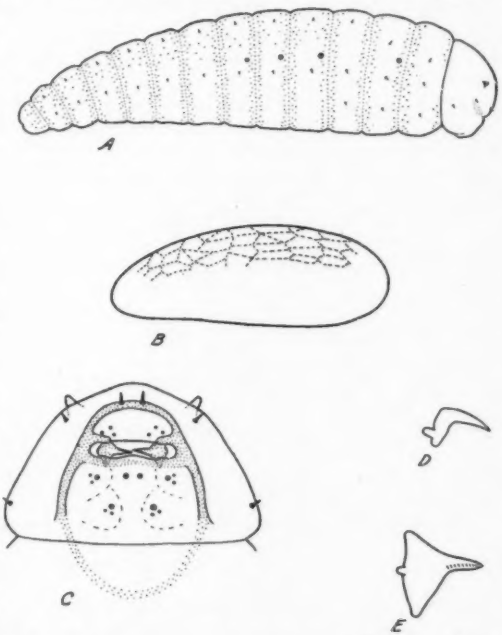

FIGURE 2.—*Schizonotus sieboldi: A*, First-instar larva, lateral view; *B*, egg; *C*, head of first-instar larva, ventral view; *D*, first-instar mandible; *E*, last-instar mandible.

LARVA

In general outline and morphological characters the larva bears a striking resemblance to the pteromalids *Dibrachys cavus* (Walk.), described by Parker (*7*), and *Habrocytus cerealellae* (Ashm.), described by Noble (*6*). It belongs to Parker's group 2.

There are five larval instars. The first and the last are distinct, but the intermediate instars differ little from one another except in size.

First Instar

The first-instar larva (fig. 2, *A*) measures about 0.45 mm in length and 0.14 mm in width. Its shape is typical of ectoparasitic chalcidoids. It has a well-defined head and 13 body segments that are about equal in length. Each segment bears a few rows of extremely minute cuticular spines on both the anterior and the posterior margins. In addition, each side bears the following sensory spines: One near the median dorsal line and one near the median lateral line of each of the first 11 segments, and one near the median ventral line of each of the first 3 segments. The sensory spines are so minute and so difficult to distinguish from the cuticular spines that the complete row along the median lateral line was not distinguished in any one specimen.

The head (fig. 2, *C*) is thimble-shaped and lightly sclerotized. The distinguishing characters are indicated in the figure. There is, however, another pair of sensory spines on the front of the head between the antennae. The mandibles (fig. 2, *D*) are simple, sickle-shaped, sclerotized hooks 0.02 mm long.

The respiratory system consists of a pair of lateral trunks united anteriorly in the first thoracic and posteriorly in the eighth abdominal segment. There are four pairs of open spiracles, a pair on the second thoracic and on each of the first three abdominal segments. They measure about 0.005 mm in diameter. Rudimentary spiracular branches are present in the third thoracic and the fourth to eighth abdominal segments, although there are no open spiracles on these segments. The spiracular branches give rise to several dorsal branches in each segment. The main ventral branches arise from the lateral trunk near the base of the spiracular branches. The head is supplied from branches arising in the first thoracic segment, and the last two abdominal segments receive branches from the longitudinal trunks in the eighth abdominal segment.

The digestive and nervous systems of this instar were not studied.

Second Instar

Second-instar larvae average 0.74 mm long and 0.38 mm wide. They are similar to the first instar, but the mandibles are shaped like those of the full-grown larva, although only 0.025 mm long. The mesothoracic spiracle is about twice as large as it is in the first instar, measuring 0.011 mm in diameter.

Third Instar

Third-instar larvae average 0.95 mm long and 0.52 mm wide. They are easily distinguished from the first two instars, for they have nine pairs of open spiracles, a pair each on the mesothoracic and metathoracic segments, and on each of the first seven abdominal segments. The mesothoracic spiracle measures 0.015 mm in diameter. The mandibles have a row of fine teeth on the inner side, and are of the same shape as in the last instar; they are 0.03 mm long.

Fourth Instar

Fourth-instar larvae differ very little from those of the preceding instar. They average about 1.40 mm long and 0.77 mm wide. The mandibles measure 0.04 mm, and the mesothoracic spiracles 0.02 mm.

Fifth Instar

The fifth-instar or full-grown larvae (fig. 3, *A*) range from 2.33 mm long and 1.64 mm wide to 1.75 mm long and 1.0 mm wide, their size depending largely upon the amount of food available. Some very small individuals complete development when several larvae parasitize one host pupa. The larva is soft and flabby. It is ovoid in shape, and the color is dull gray, since the contents of the enormous midgut show through the skin. The cuticle is very thin. As in the preceding instars, there are a few rows of minute cuticular spines on both the anterior and the posterior margins of each segment, and there are the same number of sensory spines as in the first instar.

The head (fig. 3, *B*) is similar to that of the first instar except in size. The antennae are tiny, paired, nipplelike bulges in the middle of round, slightly raised areas, the antennal foramina.[4] Just above the epistoma two small oblong areas, the dorsal tentorial marks, may be distinguished. The labium bears six pairs of tiny circular sensoria. The mandibles (fig. 2, *E*) are 0.05 mm long. Along the inner margin they bear a row of minute spines.

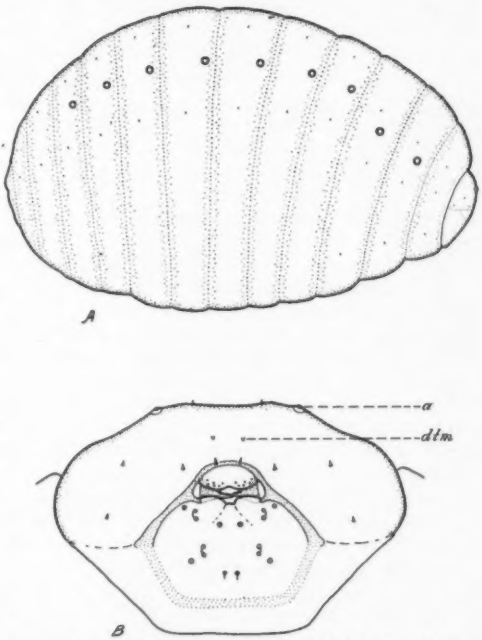

FIGURE 3.—*Schizonotus sieboldi; A*, Full-grown larva; *B*, head of full-grown larva, ventral view; *dtm*, dorsal tentorial mark; *a*, antenna.

The respiratory system is similar to that described for the first instar except that the tracheae are larger and have many more branches. There are nine pairs of open spiracles. The mesothoracic spiracle measures 0.03 mm in diameter.

The digestive and nervous systems are similar to those described for many pteromalid larvae. There is a pair of well-developed salivary glands, originating in a common duct on the floor of the mouth. This tube divides in the first thoracic segment. Each branch runs along one side of the larva, forming three wide undulations and ending at about the posterior margin of the midgut. There is a pair of straight malpighian tubes[5] with a branch running anteriorly along each side of the larva just above the salivary gland to about the second abdominal

[4] The terminology is that of Vance and Smith (*18*).

[5] In a recent article Flanders calls these tubes ileac glands. Flanders, S. E., COCOON FORMATION IN ENDOPARASITIC CHALCIDOIDS. Ann. Ent. Soc. Amer. 31: 167-180. 1938.

segment, and a short posterior malpighian tube, ending just above the rectum. The ventral nerve cord is of approximately the same width throughout, showing no pronounced thickenings at the ganglia. It terminates in the sixth or seventh abdominal segment.

The imaginal buds are well developed and easily distinguished.

The fat body is very well developed. Below the median lateral line a conspicuous double row of fat cells occurs in each segment from the third to the ninth. Above the median lateral line and in the other segments the arrangement of the fat cells does not follow the segmentation so closely. There is a narrow median dorsal and median ventral region that is not covered by the fat body except in old, fully developed larvae.

PUPA

There is no cocoon. Pupae range from 1.3 mm long and 0.7 mm wide to 2.4 mm long and 1.3 mm wide. The pupa appears entirely white when first formed, but it gradually darkens, and the mature pupa is shiny black. The thin, transparent pupal skin also darkens slightly, becoming pale yellowish brown before the adult emerges.

BIOLOGY AND HABITS

SEASONAL HISTORY

Schizonotus sieboldi overwinters as an adult, becoming active in the spring and attacking the first generation of host pupae. Two or three generations are completed during the season.

The adults probably hibernate under loose bark, in debris at the base of the tree, or in similar places. In the laboratory they usually remain inactive in small groups on the sides or in the corners of the boxes provided for them, but frequently they lodge in the crevices of the cork stoppers or between the top and sides of the box.

Observations on seasonal development were made at Melrose Highlands, Mass., in 1935 by rearing in outdoor cages. Host pupae of the first generation were collected in the field on June 17, and the first generation of *Schizonotus* adults issued from July 2 to 8. The first-generation females oviposited from July 6 to 10, and the second-generation adults issued July 22 to 24. Second-generation females oviposited from July 26 to 30, and third-generation adults issued from August 12 to 14. So few host pupae were present in the field at this late date that probably most of the third-generation adults hibernated. A very few pupae were attacked and one fourth-generation adult issued on September 2.

Probably these conditions are closely approximated in the field by at least a portion of the *Schizonotus* present. However, after the issuance of the first-generation females there is constant overlapping, because the females oviposit over a considerable period and may still be ovipositing when their first progeny appear. This is equally true of the second generation, and consequently all stages of *Schizonotus* are found in the field from the time the first-generation females appear until the end of the season.

HABITS OF THE ADULT

The adults live well in confinement when fed dry lump sugar and a honey solution (1 part honey to 5 parts water) held on absorbent cotton. Several lots totaling 69 males lived an average of 7 days,

with a minimum of 2 and a maximum of 12 days. Females live considerably longer. Two lots, totaling 30, isolated and held for notes on daily oviposition, lived an average of 27 days, with a minimum of 9 and a maximum of 41.

Schizonotus sieboldi adults mate readily in confinement. Ten pairs spent an average of 48 seconds in coitu.

The first eggs are deposited 2 or 3 days after a female emerges. Of 10 isolated females, 2 oviposited on the second and 8 on the third day after emergence. Oviposition takes place readily under laboratory conditions. The egg is laid externally, usually on the underside of the pupa. The female often paralyzes the host somewhat by inserting her ovipositor in the posterior end of the pupa, and then after 3 or 4 minutes approaches it again, this time from the front or side. Lying on her side she pushes her ovipositor, and often most of her body, under the pupa, and deposits from one to several eggs. The preferred location for eggs seems to be the thoracic region between the appendages, but often the abdomen and sometimes even the dorsum is chosen. The eggs are laid singly and are not fastened in any way, although they may adhere slightly to the host. After oviposition the female often drills a feeding puncture in the dorsum of the pupa. This sequence of behavior is often varied, and paralyzing or feeding is frequently omitted. Unmated females oviposit readily; their progeny are males.

It was noted in the field that *Plagiodera* prepupae sometimes bore eggs of *Schizonotus*, and once a well-developed *Schizonotus* larva was found on a prepupa. In the laboratory prepupae were attacked fairly readily, but a large percentage of those attacked failed to pupate, and most of those failing to pupate died without producing *Schizonotus*. Out of 103 prepupae attacked, 37 formed pupae and 66 failed to pupate. Of the 37 that formed pupae, 34 produced *Schizonotus*. Of the 66 that failed to pupate 61 produced nothing and 5 produced *Schizonotus*. The parasite may, therefore, occasionally complete its development on prepupae.

Rather extensive observations were made regarding the reproductive capacity of female *Schizonotus*. For 2 consecutive years 15 females were isolated in 4-inch glass vials and provided with fresh pupae daily. Each day the pupae were removed, and the eggs deposited were counted. The largest number of eggs laid by any one female was 424. With the exception of 1 day, she oviposited for 36 consecutive days. The largest number of eggs laid in any 1 day was 34. The previous day she had laid 6, and the following day she laid 13 eggs. When this female died, dissection showed a number of eggs in the ovarioles, but some of the follicles were empty at their extremities. The records made the first year were inadequate, because some females were living after the supply of host material was exhausted. The second year 15 females averaged 181 eggs each.

During the winter of 1936–37 observations were made on the ability of the adults to hibernate under cage conditions. Three lots were held in glass-covered wooden boxes, measuring 9 by 5 by 3½ inches. The boxes contained pieces of porous cork, in which the insects like to crawl, and each lot was supplied with food in the form of a honey-water solution held on absorbent cotton. Lot 1 consisted of 100 males and 100 females that emerged August 12. They were held in an outdoor insectary during the period of observation. First

activity was noted April 24, and an examination on May 6 showed 36 living females. All the males were dead. Between May 6 and June 8, when the first host pupa was observed in the field, 27 more females died, leaving only 9 active females. Lot 2 consisted of 200 males and 200 females that issued August 11. They were held at 62° F. until October 1 and then placed at a nearly constant temperature of 44° and a relative humidity of 70 percent. On May 6 there were 111 females living. They were then held in an inside insectary until June 8. There were 94 living on that date. Lot 3 contained 29 males and 30 females that issued the first part of July. These adults were from the first generation completed under field conditions. They were held under the same conditions as lot 2, and there were three females still alive on June 8. The heavy mortality occurring in lot 1 between May 6 and June 8 may have been caused by too close confinement during several very warm days. The insects were very active on these days, and had they been in the field they might have sought much cooler conditions. Lot 2 was held inside and was therefore not subjected to such extremes of temperature.

LARVAL DEVELOPMENT

The egg of *Schizonotus sieboldi* hatches in from 36 to 48 hours. The young larva may crawl about, but almost immediately it begins to feed on the soft underside of the host pupa. The larva feeds externally throughout its life, sucking the contents of the host pupa through the delicate skin. The rate of development depends somewhat upon the temperature, but it is usually rather rapid. From 36 to 48 hours is sufficient for each of the first four larval instars, 1 to 2 days for the last instar, 1 day for the prepupa, and about a week for the pupa. Adults usually issue in from 14 to 16 days after egg deposition, but they have been reared in 11 days in the laboratory.

NUMBER AND SEX OF SCHIZONOTUS PER HOST PUPA

Three hundred field-collected *Plagiodera* pupae isolated at the time of collection, and subsequently proved to be parasitized by *Schizonotus*, were examined for data regarding the number and the sex of the parasites. A total of 655 *Schizonotus* adults issued, or an average of slightly more than 2 per pupa. Sixty-three pupae produced only 1 parasite each, 142 produced 2, 75 produced 3, 18 produced 4, 1 produced 5, and 1 produced 6. There were 328 males and 327 females. Both sexes of *Schizonotus* were represented in most cases where more than one parasite developed on a single pupa. Out of 229 pupae, 180 produced both sexes and 49 had more than one specimen of the same sex.

SUPERPARASITISM AND SECONDARY PARASITISM

Cushman (*2*) noted that in the case of superparasitism by *Schizonotus* young larvae were found feeding on the older parasites. Such a phenomenon was not observed during this study, and it is believed to be of rather rare occurrence. As a rule several *Schizonotus* larvae feed side by side on a single host pupa. Frequently a tiny undernourished larva completes development, but it is believed that most such larvae are crowded out altogether and die of starvation.

Jones (*4*) recorded heavy parasitization of *Schizonotus* collected in the field at Milton, Mass., in 1932. Out of 84 pupae that were parasitized, *Schizonotus* issued from only 39. In the remaining 45 pupae the *Schizonotus* had been attacked by hyperparasites. Two species of hyperparasites, *Pleurotropis tarsalis* (Ashm.) and *Tetrastichus*, n. sp. (undescribed) were reared from *Schizonotus* by the writer at practically every point at which *Schizonotus* was collected. Figures on the extent of secondary parasitization were obtained from a few small collections made in 1936. They are presented in table 3.

TABLE 3.—*Secondary parasitization of field-collected Schizonotus larvae and pupae*

Place of collection	From field-collected larvae				From field-collected pupae			
	Larvae collected	Adults issued			Pupae collected	Adults issued		
		Schizonotus	*Tetrastichus*, n. sp.	*Pleurotropis tarsalis*		*Schizonotus*	*Tetrastichus*, n. sp.	*Pleurotropis tarsalis*
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Milton, Mass.	45	35	10	----------	102	65	31	2
Belmont, Mass.	54	25	23	----------	53	15	27	3
Saugus, Mass.	135	129	5	----------	148	136	12	----------
Hamden, Conn.	60	58	1	1	60	48	5	7

The data presented in table 3 indicate that at times *Schizonotus* is severely attacked by hyperparasites. Since so few collections were made in which *Schizonotus* was isolated, however, no definite conclusions regarding their importance can be made.

The life history of *Pleurotropis tarsalis* has been determined by Muesebeck and Dohanian (*5*). The species acts as both a secondary and a tertiary parasite when it attacks cocoons of *Apanteles melanoscelus* (Ratz.). It seems probable that it may develop directly upon *Schizonotus*, attacking the pupa, or upon *Tetrastichus*, if the attacked pupa already contains a *Tetrastichus* larva. It oviposits directly into its host. One or two generations are completed annually.

Tetrastichus, n. sp., also oviposits directly into its host. The parasite drills through the pupal shell of *Plagiodera* and into the *Schizonotus* larva or pupa beneath. It has also been observed ovipositing directly into a *Schizonotus* pupa. Hibernating larvae produced adults the last of May. More than one generation may develop in a season. Some first-generation larvae hibernate, but some first-generation adults issued as early as June 28 and adults of a later generation issued in September.

SUMMARY

Schizonotus sieboldi (Ratz.) is a primary parasite of the imported willow leaf beetle (*Plagiodera versicolora* (Laich.)) and some other closely allied Chrysomelidae. It is well distributed throughout Europe and the United States and is apparently present wherever the beetle occurs in this country. High percentages of parasitization have been noted over a 3-year period in the vicinity of Boston, Mass., but about the same degree of host infestation seems to have been maintained.

The immature stages of *Schizonotus sieboldi* have been described in some detail. They are very similar to those of the pteromalids *Dibrachys cavus* (Walk.) and *Habrocytus cerealellae* (Ashm.).

The winter is spent as an adult. Two and sometimes three generations develop during the summer. The females live, mate, and oviposit well under laboratory conditions. The maximum number of eggs laid by one female was 424. The eggs are laid singly on the venter of the host pupa, and the larvae feed externally. About 15 days is required from egg deposition to adult emergence. On an average two individuals develop on one *Plagiodera* pupa. The proportion of the sexes is equal.

Two hyperparasites, *Tetrastichus*, n. sp., and *Pleurotropis tarsalis* (Ashm.), have been reared from *Schizonotus* pupae. At times *Tetrastichus* is an important enemy of *Schizonotus*.

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INCIDENCE AND INHERITANCE OF POLLEN STERILITY IN THE POTATO[1]

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INTRODUCTION

Although pollen sterility effectively prevents fruiting in most of the cultivated varieties of the potato (*Solanum tuberosum* L.), seedling populations have appeared to be relatively fruitful over a period of 15 years. These observations are based on a study of the condition of seedling populations with respect to pollen viability and on tests to determine the heritability of pollen sterility.

CONDITION OF SEEDLING POPULATIONS WITH RESPECT TO POLLEN VIABILITY

INCIDENCE OF VIABLE POLLEN SELFED LINES

In 1935 and 1936 observations were made on the flowering and fruiting individuals obtained among 2,786 individuals from 101 lines selfed from 1 to 3 generations. The lines represented material of diverse origin. Of the 2,786 individuals for which data are given in table 1, 1,332 flowered and 811 set fruit. Failure to flower resulted

TABLE 1.—*Flowering and fruit production in the selfed lines grown at Castle Danger, Minn., in 1935 and 1936*

Year	Families	Individuals	Individuals—	
			Flowering	Fruiting
	Number	*Number*	*Number*	*Number*
1935	32	1,355	593	227
1936	69	1,431	739	584
Total	101	2,786	1,332	811

in absence of fruit on 50.2 percent of the total population. Presumably the 60.9 percent of flowering individuals that set fruit had viable pollen in quantities adequate for fruit setting. This leaves 39.1 percent of the flowering individuals that may have had an insufficient supply of viable pollen for fruit production. When the observations for the 2 years are examined separately, it is found that of the flowering individuals 38.3 percent fruited in 1935 and 79.0 percent in 1936. The fact that fewer fruited in 1935 was probably only partly if at all due to a greater prevalence of pollen sterility.

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It will be noted that the percentage of flowering individuals was also smaller in 1935.

TABLE 2.—*Percentage of cross-pollinations in three different localities that set fruit in 1930 and in 1932–37*

Year	University Farm		Duluth		Castle Danger	
	Cross-pollinations	Successful cross-pollinations	Cross-pollinations	Successful cross-pollinations	Cross-pollinations	Successful cross-pollinations
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1930	229	24.0	245	27.4		
1932	63	12.7	56	30.4	53	58.5
1933	36	.0	27	22.2	51	66.6
1934			198	16.2	269	45.4
1935			59	28.8	435	39.3
1936					264	46.9
1937					305	57.4

The importance of factors other than pollen sterility in restricting fruit set is indicated by the percentage of hand cross-pollinations that set fruit in different years and at three [2] locations, as shown in table 2. The percentage of hand-pollinations that set fruit ranged from 0.0 to 66.6. These extremes were obtained in 1933 on identical samples; one grown at University Farm, the other at Castle Danger. In general the percentage of hand-pollinations that set fruit was higher at Castle Danger than at Duluth, and was lowest at University Farm. These differences may have been associated with temperatures which were more favorable to the growth of the potato at Castle Danger than at Duluth and least favorable at University Farm. The results indicate that after flowers are pollinated with viable pollen, subsequent environmental conditions may influence the set of fruit. These environmental factors apparently affect the fruit set at the time of pollination and for a few days thereafter. This is indicated by the percentage of hand cross-pollinations that set fruit in each of three periods in 1936 at Castle Danger.

TABLE 3.—*Percentage of cross-pollinations that fruited in each of three periods at Castle Danger, Minn., in 1936*

Period	Cross-pollinations	Successful crosses		Clusters pollinated	Clusters setting fruit
	Number	*Number*	*Percent*	*Number*	*Percent*
July 14–22	123	64	52.0	197	43.1
July 23–31	90	53	58.9	130	54.6
Aug. 6–10	51	7	13.7	82	9.8

The data presented in table 3 show that the conditions which caused the low set of fruit from August 6 to 10, apparently had not influenced the set of fruit during the period from July 23 to 31. The percentage of hand-pollinated individuals that set fruit was lower in all three periods than the percentage of individuals that set fruit in the selfed lines in the same plot as shown in table 1 for 1936.

[2] These locations were: University Farm, St. Paul, Minn.; a plot at Duluth about 6 miles inland from Lake Superior; and plots at Castle Danger immediately adjacent to the north shore of Lake Superior and on a point extending into the lake.

The observations on the selfed lines may now be evaluated. Of the 2,786 observed individuals, 1,454 failed to flower, and of the 1,332 that flowered, 811 set fruit. From these results it would appear that 60.9 percent of the population had a supply of viable pollen adequate for natural fruit setting. The evidence presented on the influence of environmental factors in restricting fruit set on hand-pollinated flowers suggests that the failure of 39.1 percent of the flowering individuals in the selfed lines to set fruit may have been due to these factors rather than to an insufficient supply of viable pollen for natural fruit production.

INCIDENCE OF VIABLE POLLEN IN A GROUP OF CLOSELY RELATED LINES

It has been shown that while fruit setting may give some approximation of the degree of pollen sterility among the flowering individuals of a population, its value is limited by the inability to eliminate the influence of other factors that may cause nonfruiting of flowering individuals. The observations suggested the possibility that relatively few of the seedlings from seed of naturally set fruit may have had an insufficient supply of viable pollen for fruit production. To obtain information on this point a study was made of the pollen and fruit setting in 514 of 1,431 individuals from 69 families representing the F_1 to the F_4 generations of a cross. The condition of the pollen was recorded in percent stainable with acetocarmine. It is probably safe to conclude that pollen which does not stain is nonfunctionable. The association between stainable pollen and fruit production is presented in table 4.

TABLE 4.—*Association between the average percentage of stainable pollen and fruiting in 514 seedlings from 69 families constituting the F_1 to the F_4 generation, inclusive, of a cross*

Stainable pollen (percent)	Plants observed	Plants setting fruit		Stainable pollen (percent)	Plants observed	Plants setting fruit	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
0.0–5.0	60	33	55.0	60.1–70.0	80	76	95.0
5.1–10.0	25	19	76.0	70.1–80.0	53	52	98.1
10.1–20.0	42	33	78.6	80.1–90.0	26	26	100.0
20.1–30.0	38	29	76.3	90.1–100.0	1	1	100.0
30.1–40.0	44	35	79.5				
40.1–50.0	64	60	93.7	Total or average	514	439	85.4
50.1–60.0	81	75	92.6				

Of the 60 seedlings that had between 0 and 5 percent stainable pollen, 55 percent set fruit, and of the 25 seedlings that had between 5.1 and 10 percent of stainable pollen, 76 percent set fruit. There was a gradual increase in the number of individuals setting fruit as the percentage of stainable pollen increased. While the association between amount of stainable pollen and the number of fruiting individuals found in the two lower classes was not unexpected, the significance of the association in the higher classes is not clear. The fruiting observed in individuals of the group with 0 to 5 percent stainable pollen suggests that under the conditions of these observations most of the stainable pollen was probably viable.

It will be noted that 439 of the 514 individuals, or 85.4 percent, set fruit. This is slightly higher than the 79.0 percent obtained in the selfed lines in 1936. Since 33 of the 60 individuals having between

0 and 5 percent stainable pollen set fruit it would appear that 27 individuals, or 5.3 percent of the total population, may have had an insufficient supply of viable pollen for fruit setting. The observations tend to support the view that pollen sterility plays a relatively minor role in restricting fruiting in seedlings from seed of naturally set fruit.

The influence of environmental factors on the viability of pollen is indicated by the percentage of stainable pollen observed in 10 clones, for each of two periods (table 5). A significant difference in viability is indicated between the two periods for all but one clone. The decrease in stainable pollen noted in the period from June 26 to July 5 was associated with a distinct rise in temperature and a large amount of flower abscission. The data are in harmony with the observations of Stow [3] and support his view that pollen viability is modified by temperature.

TABLE 5.—*Stainable pollen observed in 10 clones during two periods in 1933 at University Farm, Minn.*

Variety or stain of clone	Stainable pollen observed from—		Variety or stain of clone	Stainable pollen observed from—	
	June 12 to 21	June 26 to July 5		June 12 to 21	June 26 to July 5
	Percent	*Percent*		*Percent*	*Percent*
Green Mountain	1.8	0.6	11–1–12	24.0	18.8
Katahdin	31.4	11.5	40–2–2	23.5	2.3
4–20–1	42.5	47.4	41–1–1–8–1	26.8	10.2
4–39–1–1	17.2	.0	66–5	26.0	.2
5–4–1–2	24.2	.0	*Solanum demissum*	17.6	1.2

It has been observed that clones may vary in percentage of stainable pollen between periods within a season, the amount of variation depending upon environmental conditions. Significant variation between paired flowers of a clone have also been observed. Table 6 gives the percentage of stainable pollen observed in each of two concurrently developing buds on each of four clones. The percentages are based on 2,500 observed pollen grains in each flower. While the variation between flowers of the same clone is distinctly less than that between clones, the differences between paired flowers is significant for Selections 1 and 3. These results are in agreement with those of Longley and Clark,[4] who mention numerous instances of observed variation in tetrad formation between flower buds of the same clone.

The variations observed suggest the desirability in inheritance studies of making progeny tests in classifying the individual segregates.

TABLE 6.—*Percentage of stainable pollen in two paired, freshly opened flowers from each of four clones*

Flower No.	Selection 1	Selection 2	Selection 3	Chippewa
	Percent	*Percent*	*Percent*	*Percent*
1	2.5	34.8	35.0	0.0
2	12.3	32.7	20.8	.1

[3] STOW, ISAMU. A CYTOLOGICAL STUDY OF POLLEN STERILITY IN SOLANUM TUBEROSUM L. Japan. Jour. Bot. 3: [217]–238, illus. 1927.

[4] LONGLEY, A. E., and CLARK, C. F. CHROMOSOME BEHAVIOR AND POLLEN PRODUCTION IN THE POTATO. Jour. Agr. Research 41: 867–888, illus. 1930.

INHERITANCE OF POLLEN STERILITY

In 1924 a cross was made between the Lookout Mountain variety and an unknown variety found as a mixture in a commercial field of Irish Cobbler. Both parents were known to have a moderate amount of fertile pollen. The F_1 plants were relatively fruitful and seed of 45 families of the F_2 to the F_4 generations was accumulated during a period of 13 years. The 45 families were grown in 1937 and the percentage of stainable pollen of a number of individuals in each family was determined.

With respect to their production of stainable pollen, the 16 individuals of the F_1 family fell naturally into 3 groups, as follows: 5 with 0 to 17 percent of stainable pollen, 6 with 30 to 46 percent, and 5 with 70 to 77 percent. Three types of segregation were obtained from the F_1 plants. Four gave progenies ranging in percentage of stainable pollen from high to medium. One appeared to be segregating for high, medium, and low, and two for medium and low. The percentage of stainable pollen observed in each plant is given in table 7. It will be noted that in the progeny of F_1 plant 5–8, there is

TABLE 7.—*Types of segregation for percentage of stainable pollen obtained from F_1 plants from a cross of Lookout Mountain × Minnesota Accession 123*

F_1 plant No.	Stainable pollen in F_2 segregate No.—															Individuals	Mean percentage
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Number*	
5-2	80	69	65	65	64	62	59	59	57	54	52	45	41	36	----	14	57.7
5-10	86	83	81	77	63	49	42	40	25	15	----	----	----	----	----	10	56.1
5-3	78	72	58	56	51	41	29	20	----	----	----	----	----	----	----	8	50.6
5-8	72	70	63	59	59	58	56	51	47	40	40	33	30	29	0	15	47.1
5-16	65	55	38	38	14	1	----	----	----	----	----	----	----	----	----	6	35.2
5-5	73	18	12	9	7	3	2	0	0	0	----	----	----	----	----	10	12.4
5-4	37	22	9	5	4	2	0	0	0	0	----	----	----	----	----	10	7.9

one plant with no stainable pollen. It is possible that this F_1 plant was segregating for high, medium, and low stainable pollen plants. It will also be noted that a plant from 5–5 gave a high percentage of stainable pollen as compared with the other segregates observed in the family. The three types of segregation observed in the F_2 families harmonize with the observed natural grouping of the F_1 phenotypes. The F_3 and F_4 families are ranked in table 8 according to their mean percentage of stainable pollen.

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TABLE 8.—*Types of segregation for percent of stainable pollen obtained in F_3 and F_4 families from a cross of Lookout Mountain × Minnesota Accession 123*

Group	Parent No.	Stainable pollen in progeny No.—																	Individuals	Mean percentage stainable pollen
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	
1	5-10-1-5	89	85	82	78	76	74	64	63										8	76.4
	5-4-1-1	79	71	71	70	70	69												6	71.7
	5-10-2	86	83	83	83	73	70	69	68	61	48	45							11	69.9
	5-14-5	91	89	79	57	17													5	66.6
	5-3-1	80	78	76	62	60	60	59	59	58									9	65.8
	5-14-5-1	83	77	72	58	57	54	28											7	61.3
	5-14-15	85	71	65	59	57	53	50	50	44									9	59.3
	5-14-16	72	67	59	51	47													5	59.2
	5-15-1-4-1	85	74	70	65	1													5	59.0
	5-10-1-3	86	73	55	48	30													5	58.4
	5-10-1-4	91	86	83	83	83	79	79	70	61	60	22	7	4	0				14	57.7
	5-10-3-23	76	58	56	56	54	49	5											7	50.6
	5-6-1-1	87	84	76	68	65	56	56	54	51	30	23	20	16	13				14	49.9
	5-4-3-6	67	63	62	6														4	49.5
	5-14-2	74	73	61	56	55	42	38	35	0									9	48.2
	5-1-1	75	70	69	67	60	56	56	54	53	53	40	38	29	21	17	11		16	48.1
	5-2-5	64	58	37	32														4	47.7
2	5-10-3	80	63	36	11														4	47.5
	5-10-1	77	65	65	61	60	50	48	47	46	42	35	34	34	28	15			15	47.1
	5-2-4	57	46	35															3	46.0
	5-14-13	64	63	62	60	52	41	33	27	11									9	45.9
	5-11-1-4	76	71	67	10	1													5	45.0
	5-10-1-1	65	65	62	56	56	53	53	51	51	50	49	47	29	29	21	17	5	17	44.6
	5-8-2	72	67	66	58	55	52	48	48	45	40	34	18	13	2	0			15	41.2
	5-8-1	71	63	48	48	45	40	40	39	39	34	25	22	21					13	41.2
	5-2-1	74	73	56	15	13	2												6	37.8
	5-14-9	77	65	54	15	13	2												6	37.7
	5-6-2	83	75	69	69	65	53	41	31	31	13	10	2	0	0	0			15	36.1
	5-4-1	61	50	47	43	22	8	7											7	34.0
	5-3-2	64	55	54	45	16	4	0											7	34.0
3	5-4-3-4	80	70	52	51	41	40	32	31	29	29	12	12	11	6	2			15	33.2
	5-14-17	66	58	47	23	3	0												6	32.8
	5-7-2	56	54	52	48	43	41	33	25	23	22	13	5	2	2				14	29.9
	5-14-14-1	62	57	23	19	18	0												6	29.8
	5-14-12	59	51	38	34	15	6	0											7	29.0
	5-14-8	60	48	34	11	8	6												6	27.8
4	5-6-1	70	51	45	45	29	25	20	13	8	2	1	0	0	0	0			15	20.6
	5-4-3-5	58	24	9	4	1													5	19.2

There are three distinct breaks in the frequencies of the means, as follows: between 20.6 and 27.8, 37.8 and 41.2, and 50.6 and 57.7. These breaks divide the 38 families into 4 groups. The segregation observed within and between these groups suggests the possibility that this may be a natural grouping and that the parents of the 38 families may have been distributed among 4 different genotypes with respect to sterility.

The results obtained from the cross suggest that pollen sterility may be inherited as a simple mendelian character. A behavior similar to that observed might be expected if inheritance in the potato is tetrasomic and a pollen lethal lacking dominance is present. Lunden [5] has supplied evidence indicating that inheritance in the potato is tetrasomic. Assuming a pollen lethal gene designated as *V* the possible types of gametes are *V*2, *Vv*, and *v*2. If the *v*2 pollen aborts, the possible genotypes are *V*4, *V*3*v*, *V*2*v*2, and *Vv*3.

Both parents of the cross were able to produce some viable pollen. Accession 123 appeared more pollen-fertile than Lookout Mountain, for it was more effective as a male parent and selfed lines of it were easier to obtain. If Accession 123 was a *V*2*v*2 plant and Lookout Mountain a *Vv*3 plant, then the three types of segregates obtained in the F_1 could be expected. The five segregates having 70–77 percent stainable pollen would be classed as *V*3*v* plants, the six having 30–46 percent stainable pollen as *V*2*v*2 plants, and the five having 0–17 percent stainable pollen as *Vv*3 plants. It will be noted from the percentage of stainable pollen observed in the plants classed as *Vv*3 that in addition to the *v*2 gametes, from 66 to 100 percent of the *Vv* gametes aborted. In the F_2 from these F_1 plants three types of segregation would be expected, and as will be seen in table 7, the F_1 plants divide naturally into three groups, based on the mean percentage of stainable pollen in their progenies. The segregation in the families, despite the small number of individuals observed, is suggestive of the types that would be expected. In the F_3 and F_4, as will be noted in table 8, the 38 families are separated into 4 groups by more or less distinct breaks in the frequencies of their mean percentage of stainable pollen. These percentages are 76.4 to 57.7 in the first group, 50.6 to 41.2 in the second, 37.8 to 27.8 in the third, and 20.6 to 19.2 in the fourth. These groups may represent segregation from the four possible genotypes as shown in table 9. In the first group all the grains would be expected to be potentially viable, although only 64.1 percent were stainable. It is possible that the shrunken grains observed in this group would have been viable except for nutritional or other causes arresting development. In the lowest group, which presumably is composed of families derived from *Vv*3 plants, the average mean percentage of stainable pollen for the two families is 19.9. This result would be expected if all the *vv* pollen, and about 80 percent of the *Vv* pollen, aborted. This is in harmony with the behavior of the *Vv*3 plants observed in the F_1, in which the percentage of stainable pollen in different plants ranged from 0 to 17 percent, showing that none of the *vv* pollen and only from 0 to 34 percent of the *Vv* pollen was stainable.

[5] LUNDEN, AKSEL P. INHERITANCE STUDIES IN THE POTATO (SOLANUM TUBEROSUM L.). Norges Landbrukshøiskoles Åkervekstforsok Årsmeld. (1934–35) 46: 1–156. 1937.

TABLE 9.—*Probable genotype of parents of each group, the gametes and the expected segregates with the expected average percent of viable pollen from the progeny and the percent of stainable pollen observed, for each group* [1]

Group No.	Parents	Gametes	Segregates				Segregation of male gametes in progeny	
			1	2	3	4	Viable gametes calculated [1]	Stainable pollen obtained
							Percent	*Percent*
1	*V*4	*V*2	*V*4				65.00	64.1
2	*V*3*v*	*V*2 *Vv*	13 *V*4	17 *V*3*v*	4 *V*2*v*2		48.95	46.6
3	*V*2*v*2	*V*2 4 *Vv* *v*2	13 *V*4	68 *V*3*v*	77 *V*2*v*2	16 *Vv*3	33.08	32.9
4	*Vv*3	*Vv* *v*2			*V*2*v*2	*Vv*3	17.08	19.9

[1] Calculated on the basis that 35 percent of the pollen with *V*2 gametes failed to develop, and that all the *v*2 gametes and 80 percent of the *Vv* gametes resulted in aborted pollen.

The fact that only 65 percent of the *V*2 pollen from *V*4 plants, and about 20 percent of the *Vv* pollen and none of the *v*2 pollen from *Vv*3 plants was stainable, gives a basis for calculating the expected mean percentage of stainable pollen in the progenies from *V*3*v* and *V*2*v*2 plants. The calculated and the observed percentages are given in table 9 and, as will be seen, the agreement is reasonably good.

DISCUSSION

In contrast to the condition prevailing in cultivated varieties of potato, the viability of the pollen in most of the hybrids of the cross studied was relatively high. Pollen from 514 of the 1,431 individuals in the cross was examined and only 60 were found to have less than 5 percent of stainable pollen. Since 33 of these 60 produced fruit, only 27 individuals, or about 5 percent of the individuals that produced flowers, can be suspected of having failed to fruit because of an insufficient supply of viable pollen. The behavior of the F_2, F_3, and F_4 families was very similar to that observed in families derived from selfing varieties and clones of very diverse origin. This general behavior in the potato suggests that the cultivated varieties, which have little or no viable pollen, may not be representative of the species with respect to production of viable pollen. It is possible that there is sufficient antagonism between fruit production and growth of vegetative organs in the potato so that selection for high yield of tubers has tended to eliminate the profuse blooming and highly fruitful types.

It should be remembered that pollen abortion is only one of the many factors responsible for low fruiting in the cultivated varieties. Some of the other factors that influence fruitfulness in cultivated varieties are restricted habit of bloom, rudimentary to weak inflorescence, ready abscission of flowers, and poorly developed stamens. Their presence lends support to the view that selection for high yield has favored the retention of genes adversely influencing sexual reproduction. On the other hand, continued sexual reproduction tends to eliminate these genes and selection is required to retain them.

SUMMARY

Observations on 2,786 individuals derived from 101 selfed lines of potato indicated that 47.8 percent flowered and 60.9 percent of the flowering individuals set fruit. Evidence from hand cross-pollinations suggests that the failure of 39.1 percent of flowering individuals to set fruit might possibly have been due to environmental factors.

Observations on pollen and fruit setting for 514 individuals of 69 families showed an association between amount of stainable pollen and number of individuals within the group that set seed. It was calculated that on the basis of the observations 94.7 percent of the total population had viable pollen in adequate quantities for setting fruit naturally. An association was observed between percentage of stainable pollen and changes in environmental conditions.

In studying the inheritance of pollen sterility, stainability was used to differentiate between viable and nonviable pollen. The study was confined to one cross. Progeny tests were used to classify the individual segregates. The segretation obtained may be explained by assuming the presence of a tetrasomic gene which, when present in the pollen in the homozygous condition, is lethal and in the heterozygous condition partly lethal.

SEASONAL VARIATION IN THE COMPOSITION OF BLUEBUNCH FESCUE[1]

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INTRODUCTION

Bunch grasses have long been considered superior for fall and winter feeding, presumably because of the fact that there is little loss of nutrients during that period. There is, however, considerable variation among bunch grasses in composition and feeding value. This work was planned to show the chemical change throughout the year due to the effects of stage of maturity and weathering as an indication of the feeding value of a grass typical of the ranges in the State of Washington. The study was conducted with bluebunch fescue (*Festuca idahoensis* Elmer) (sometimes called blue bunch grass), because it is one of the most common range grasses of Washington, and because a typical stand of this grass was available.

REVIEW OF LITERATURE

SEASONAL VARIATION IN THE ORGANIC NUTRIENTS OF GRASSES

Much of the work done on the seasonal variation in composition of grasses has been with cultivated pasture grasses rather than with range grasses. Although the trend is probably slightly different in tame grasses, some of the work on these grasses is presented for comparison.

Woodman, Blunt, and Stewart (*23*)[3] reported that the nutritive value of pasture grass as indicated by chemical analysis is highest during the early part of the season, and as the plant matures, there is a gradual decline in nutritive value. Catlin (*5*) observed a similar decline in experiments with range grasses and other forages. Cundy (*6*) found that the decline is not usually quite so great in range grasses as in pasture grasses.

Considerable importance attaches to the seasonal trend in chemical composition of a forage as the amount of crude protein, calcium, and phosphorus in a plant is one of the best indexes of its feeding value according to Guilbert (*11*). Lush (*17*) observed that the season of the year and the rate of growth were of more importance than the type of vegetation in determining the crude protein and crude fiber of tame grasses. Hart and his coworkers (*13*) concluded that the dry matter of range forages varies from a protein-rich concentrate during the early vegetative stages to a poor roughage during the drought period.

Green (*10*) proved that the decline in protein content of grasses levels off at maturity, that there is little further decline, and in some

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[3] Italic numbers in parentheses refer to Literature Cited, p. 615.

cases a slight increase is observed throughout the fall and winter. He believes that the seasonal decline in crude protein does not represent an actual loss of this nutrient but may be attributed to the accumulation of greater quantities of crude fiber and other nutrients.

Capen and Le Clerc (*4*) reported that the crude fiber of tame pasture grass and range grass usually shows a seasonal trend in the opposite direction from that of crude protein.

Hopper and Nesbitt (*14*) observed that the seasonal variations in the crude-fat content of range grasses are small and exhibit little consistency. Cundy (*6*) learned that a majority of range grasses show an increase in crude fat as the season advances. Knight and his coworkers (*15*) found the reverse to be true.

Woodman and Oosthuizen (*25*) concluded that as a general rule there seems to be a slight increase in the nitrogen-free extract content of grasses as the season advances. However, Guilbert, Mead, and Jackson (*12*) found that there was a loss of nitrogen-free extract because of weathering of range grasses and forages in California.

Woodman, Norman, and French (*24*) suggested the following effects of drought on tame grasses: (1) A decided decrease in crude protein; (2) a slight increase in crude fiber and nitrogen-free extract; (3) a sharp increase in lime; (4) decreases in phosphorus; (5) pronounced moisture decreases; and (6) a decided decline in digestibility and nutritive value.

Archibald and Bennett (*2*) noticed that the amount of growth and percentage of crude protein and phosphorus in range and pasture grasses follow the rainfall curve very closely.

In a moist, "quick-growing" season, Woodman, Norman, and French (*24*) observed a greatly enhanced vegetative activity in pasture grasses. The amount of crude fat was depressed markedly, crude protein slightly, and crude fiber raised considerably. Knight, Hepner, and Nelson (*16*) stated that the composition of a species of range forage plant varies from year to year in the same location.

According to Hopper and Nesbitt (*14*) some of the factors that influence the chemical composition of a grass are (1) stage of growth, (2) amount of moisture, (3) fertility of soil, and (4) elevation. Knight, Hepner, and Nelson (*16*) considered the amount of sunshine, the temperature, and environmental conditions in preceding years other important factors. Archibald (*1*) added length of day to the list of factors affecting the chemical composition of grass.

According to studies by McCreary (*19*) the fineness or coarseness of the stems of range forages has no influence on the seasonal change in composition. Fagan and Milton (*8*) concluded that the change in composition of tame grasses is largely accounted for by the proportion of stem to leaf which gradually increases with age. They also observed that the leaf at all periods is richer in crude protein and lower in crude fiber than the stem.

McCreary (*18*) analyzed range grasses in late fall after the plants were in a dormant state and discovered that their feeding value as indicated by chemical analysis is little better than that of oat straw.

SEASONAL VARIATION IN THE INORGANIC NUTRIENTS OF GRASSES

McCreary (*19*) reported a gradual percentage increase of ash and lime in range grasses as the season advanced, but he suggested that it was probably due to an accumulation of dust. Roberts (*20*) observed

that approximately two-thirds of all samples of range grasses studied over a 3-year period showed an increase in ash content as the season advanced. Watkins (*22*) analyzed range grasses in the fall and again in late winter and found that there was an average loss of about 78 percent of phosphorus and 26 percent of calcium at the latter date.

Guilbert (*11*) observed extreme seasonal variations in the content of calcium and phosphorus of range forages, and calcium-phosphorus ratios as wide as 45 to 1.

An observation by Harper and Daniel, reported by Watkins (*22*), is that after a growing season with heavy rainfall, the prairie hay of Oklahoma was low in calcium and high in phosphorus, but that after a growing season with light rainfall the reverse was true. McCreary (*19*) found a very close relationship throughout the year between the percentage of phosphorus and crude protein in range grasses.

Scott (*21*) reported that precipitation apparently had no effect upon the mineral content of range grasses and forages. However, Daniel and Harper (*7*) observed that soil moisture during the growing period is an important factor in increasing or retarding the intake of calcium or phosphorus from the soil by range plants.

According to Forbes and his coworkers (*9*) the leaves of grasses are richer in calcium than the seeds, and the seeds are richer in phosphorus than the leaves. Fagan and Milton (*8*) reported that the calcium content of leaves increases with age, whereas the reverse is true of stems.

EXPERIMENTAL MATERIALS AND METHODS

The samples of bluebunch fescue were collected every 2 weeks from an area about 100 feet square midway of a south slope on a virgin bunch-grass plot of the Washington State Agricultural Experiment Station farm. The vegetation here seemed to best represent the climax type. The soil is a deep Palouse silt loam and is well drained.

The cuttings of grass were made with a knife just above the crown of the tuft. Special care was taken to include all of the basal leaves. These cuttings did not exactly represent the way in which grass would be grazed on the range but were the closest possible approach to the true condition. Seven to ten bunches were included in each sample for uniformity. Succeeding cuttings were always taken from different bunches so that each cutting represented the cumulative growth up to that time. The first new-growth sample was obtained by clipping off all the previous year's growth the preceding fall so that the sample taken was all new growth. Official analytical procedure as recommended by the Association of Official Agricultural Chemists (*3*) was followed.

SEASONAL VARIATION IN ORGANIC NUTRIENTS

The nutrient exhibiting the greatest seasonal change in chemical composition as recorded in table 1 was crude protein. In the spring of 1932 the first sample of new grass collected on April 1 contained 25.55 percent of crude protein on a water-free basis, whereas only 2.85 percent of the sample of August 7, 1931, was crude protein.

In the 4 years of this study, the decline in percentage of crude protein continued with one exception until about the first part of August to the first part of September depending upon maturity. In 1934 there was a slight increase in crude protein in the second sample of new

growth, but there was a gradual decrease in succeeding samples. At the point, when the content of crude protein leveled off, the grass was quite dry, and the seeds were almost completely shattered out. In the fall of 1932 the decline lasted a little longer than usual, probably because of the small amount of precipitation in the early fall as shown in table 2. However, growing conditions had been quite favorable in 1932, and the percentage of crude protein at the low point was higher than in the other years of the study.

TABLE 1.—*Chemical composition of semimonthly samples of bluebunch fescue (Festuca idahoenesis Elmer) (water-free basis)*

Date of sampling	Ash	Crude protein (N×6.25)	Carbohydrates		Crude fat	Calcium	Phosphorus	Ca as 1 ratio (Ca as 1)
			Crude fiber	N-free extract				
1930	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Oct. 3	18.10	3.75	30.58	44.14	3.43	0.22	0.12	0.54
Oct. 17	18.56	4.40	30.29	43.78	2.97	.24	.15	.62
Oct. 31	19.12	3.98	31.05	42.90	2.95	.27	.14	.52
Nov. 17	19.12	4.05	31.83	41.84	3.16	.28	.13	.46
Dec. 3	19.38	4.08	30.77	42.81	2.96	.27	.16	.59
Dec. 12	19.17	4.27	31.22	42.07	3.27	.30	.13	.43
Dec. 29	18.75	4.47	30.13	43.12	3.53	.33	.12	.36
1931								
Jan. 9	18.98	4.40	30.79	42.06	3.77	.32	.11	.34
Jan. 23	18.73	4.87	30.90	42.25	3.25	.33	.12	.36
Feb. 6	19.65	5.25	30.45	41.85	2.80	.31	.14	.45
Feb. 20	19.96	4.52	31.43	41.50	2.59	.30	.13	.43
Mar. 6	19.38	5.35	30.08	42.82	2.37	.28	.15	.54
Mar. 20	18.22	4.56	32.38	43.07	1.77	.27	.13	.48
Do.[1]	9.08	18.65	23.48	44.66	4.13	.44	.46	1.04
Apr. 3	9.39	16.07	24.76	46.48	3.30	.37	.38	1.03
Apr. 17	9.53	13.61	24.60	48.17	4.09	.38	.35	.92
May 1	13.86	13.09	28.31	40.95	3.79	.39	.38	.97
May 15	12.97	11.20	30.20	42.19	3.44	.37	.34	.92
May 29	10.12	8.93	33.86	44.40	2.69	.30	.30	1.00
June 12	8.29	6.01	33.85	49.30	2.55	.24	.20	.83
June 26	9.24	5.99	32.00	49.76	2.99	.30	.22	.73
July 10	8.85	3.65	35.91	49.13	2.46	.22	.15	.68
July 24	10.24	3.18	34.53	49.23	2.82	.25	.16	.64
Aug. 7	10.74	2.85	34.06	49.75	2.60	.25	.15	.60
Aug. 21	11.60	3.10	33.75	48.76	2.79	.26	.16	.62
Sept. 4	12.69	3.27	30.29	50.87	2.88	.23	.14	.61
Sept. 18	10.98	2.97	32.72	50.94	2.39	.19	.09	.47
Nov. 27	11.48	3.58	35.10	47.35	2.49	.24	.11	.46
Dec. 11	14.05	4.19	33.41	45.50	2.85	.26	.09	.35
Dec. 25	18.80	4.92	31.19	43.22	1.87	.33	.10	.30
1932								
Jan. 8	16.83	4.50	33.60	43.34	1.73	.27	.10	.37
Jan. 22	17.62	4.20	32.49	43.14	2.55	.29	.10	.34
Feb. 5	14.64	3.75	35.74	44.43	1.44	.29	.08	.28
Feb. 19	12.85	3.19	37.84	44.52	1.60	.26	.07	.27
Mar. 5	19.21	4.85	31.73	42.34	1.87	.34	.11	.32
Mar. 18	19.13	5.06	31.76	41.73	2.32	.32	.12	.38
Apr. 1	19.18	5.08	30.95	42.27	2.52	.33	.11	.33
Do.[1]	10.24	25.55	22.83	37.41	3.97	.48	.64	1.33
Apr. 15	11.48	18.72	22.42	43.13	4.25	.41	.27	.66
Apr. 29	12.95	15.92	26.43	40.69	4.01	.39	.33	.85
May 13	13.80	12.17	27.67	42.56	3.80	.39	.32	.82
May 27	12.83	9.54	27.19	46.93	3.51	.37	.25	.68
June 10	15.71	9.16	28.70	42.64	3.79	.41	.23	.56
June 24	15.50	7.42	29.24	44.72	3.12	.40	.21	.52
July 8	16.83	5.40	29.41	45.24	3.12	.37	.18	.49
July 22	15.14	4.70	29.85	46.17	4.14	.35	.18	.51
Aug. 5	14.79	4.56	29.69	46.95	4.01	.34	.15	.44
Aug. 19	16.32	4.70	28.26	47.14	3.58	.39	.19	.49
Sept. 2	16.08	4.51	27.03	48.17	4.21	.44	.22	.50
Sept. 16	15.08	4.61	27.37	48.85	4.09	.40	.18	.45
Sept. 29	14.73	4.48	27.99	48.52	4.28	.40	.16	.40
Oct. 14	17.38	4.06	27.82	46.63	4.11	.33	.10	.30
Oct. 28	14.72	4.34	29.66	46.88	4.43	.36	.17	.47
Nov. 18	15.96	4.21	31.18	44.48	4.17	.37	.16	.43
Nov. 25	16.14	4.86	31.01	44.10	3.89	.36	.14	.39
Dec. 12	16.58	4.76	31.17	44.12	3.37	.36	.14	.39
Dec. 22	15.91	4.52	31.45	44.98	3.14	.27	.14	.52

[1] New growth here to end of year.

TABLE 1.—*Chemical composition of semimonthly samples of bluebunch fescue (Festuca idahoensis Elmer) (water-free basis)*—Continued

Date of sampling	Ash	Crude protein (N×6.25)	Carbohydrates		Crude fat	Calcium	Phosphorus	Ca as 1 ratio (Ca as 1)
			Crude fiber	N-free extract				
1933	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Jan. 6	18.24	4.93	30.52	43.00	3.31	.32	0.16	0.50
Jan. 20	17.18	5.31	31.83	42.05	3.63	.36	.13	.36
Feb. 3	16.53	5.76	31.93	42.84	2.94	.33	.13	.39
Feb. 17	18.42	5.16	31.22	41.82	3.38	.35	.14	.40
Mar. 3	17.87	5.73	31.51	41.83	3.66	.37	.14	.38
Mar. 17	18.13	5.35	31.68	42.34	2.50	.35	.18	.51
Mar. 31	17.98	5.18	30.71	42.77	3.38	.35	.15	.43
Apr. 14 [1]	11.61	19.80	21.23	43.59	3.77	.44	.49	1.11
Apr. 28	13.52	14.90	27.40	40.14	4.04	.40	.41	1.02
May 12	12.11	12.44	25.80	46.00	3.65	.37	.38	1.03
May 26	11.09	10.62	30.10	43.94	3.45	.36	.28	.78
June 9	13.64	9.29	32.64	40.99	3.44	.34	.24	.70
June 23	13.58	6.93	30.38	46.03	3.08	.36	.24	.67
July 7	14.00	5.92	30.75	46.53	2.80	.34	.21	.62
July 23	13.86	5.46	32.01	45.54	3.13	.45	.24	.53
Aug. 4	12.50	4.11	31.97	48.36	3.12	.35	.17	.48
Aug. 18	13.68	4.38	33.27	44.91	3.76	.37	.16	.43
Sept. 1	14.10	4.30	31.03	49.19	1.38	.27	.16	.59
Sept. 16	12.66	3.63	32.56	47.61	3.54	.23	.15	.65
Oct. 1	14.36	4.37	33.22	45.09	2.96	.27	.15	.56
Oct. 13	14.26	5.29	30.01	46.74	3.70	.26	.20	.77
Oct. 27	13.95	5.84	31.63	44.74	3.84	.29	.18	.62
Nov. 10	12.62	4.53	34.15	46.37	2.33	.23	.14	.61
Nov. 24	12.27	4.32	34.88	46.38	2.15	.30	.15	.50
Dec. 8	12.01	4.51	34.91	45.88	2.69	.27	.14	.52
1934								
Jan. 5	10.82	5.11	35.45	45.82	2.82	.24	.19	.79
Jan. 19	12.07	4.56	35.65	45.23	2.49	.20	.17	.85
Feb. 2	10.20	4.51	36.14	47.16	1.99	.22	.14	.64
Feb. 16	15.02	6.73	30.85	44.34	3.06	.30	.21	.70
Mar. 2	14.10	8.90	29.48	44.06	3.46	.31	.25	.81
Do. [1]	9.96	17.40	25.96	42.38	4.30	.38	.44	1.16
Mar. 16	10.67	19.26	24.68	41.25	4.14	.37	.48	1.30
Mar. 30	11.45	15.14	27.58	42.01	3.82	.37	.42	1.14
Apr. 13	11.86	12.66	27.80	44.10	3.58	.38	.40	1.05
Apr. 27	12.45	11.13	31.15	42.89	2.40	.41	.37	.90
May 11	12.76	8.75	29.15	46.30	3.04	.42	.30	.71
May 25	13.59	7.75	29.63	45.71	3.32	.43	.28	.65
June 8	14.74	6.76	29.68	45.10	3.72	.44	.26	.59
June 22	14.63	5.82	29.39	46.50	3.86	.41	.23	.56
July 6	16.35	5.18	30.46	44.18	3.83	.46	.23	.50
July 20	15.47	5.14	29.08	46.46	3.85	.39	.22	.56
Aug. 3	16.65	4.76	29.12	45.72	3.75	.44	.21	.48
Aug. 17	15.72	4.36	28.24	47.52	4.16	.45	.19	.42
Aug. 31	14.91	4.00	29.55	47.19	4.35	.40	.17	.42
Sept. 14	15.60	3.47	29.30	47.99	3.64	.40	.15	.38
Sept. 29	14.57	4.13	30.86	46.97	3.47	.40	.13	.32
Oct. 15	17.63	4.67	27.87	46.55	3.28	.45	.16	.36
Oct. 29	17.68	4.52	28.79	45.32	3.69	.39	.12	.31
Nov. 10	17.55	4.84	29.38	44.89	3.34	.40	.14	.35

[1] New growth here to end of year.

The percentage of crude protein consistently held its own with the advent of the fall rains and usually increased toward the latter part of the winter. On account of the extremely mild winter of 1933–34 (table 3) and the exceptionally heavy precipitation early in the winter, there was more growth of new grass in late winter than usual. Because of the great difficulty of removing all of the tips of the green grass without losing some of the leaves of the old grass, a small amount was included in the clippings taken during the month preceding the first new growth sampling. This is probably the main reason for the percentage increase in crude protein just before the first new grass was collected on March 2, 1934. Increases in crude protein earlier in the winter were probably caused largely by decreases in nitrogen-free extract.

TABLE 2.—*Precipitation by months for the years in which the study was conducted, and 5-year averages by months, 1930–34*

Month	1930	1931	1932	1933	1934	5-year average
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
January	1.90	2.30	2.66	4.48	2.77	2.82
February	3.38	2.02	1.80	3.15	.56	2.18
March	2.79	4.55	5.06	1.84	2.97	3.44
April	1.87	1.98	.85	.70	.88	1.26
May	2.61	.44	1.96	.85	1.49	1.47
June	.94	1.34	.24	.89	2.68	1.22
July	.05	.00	.31	.30	.19	.17
August	.04	.00	.15	.60	.03	.16
September	.94	.70	.17	1.55	.64	.80
October	1.05	1.38	2.41	3.46	2.64	2.19
November	2.49	2.08	5.00	1.67	1.85	2.62
December	1.36	3.05	1.48	7.12	2.60	3.12
Total	19.42	19.84	22.09	26.61	19.30	21.45

TABLE 3.—*Temperatures by months for the years in which the study was conducted and 5-year averages by months, 1930–34*

Item	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average
1930	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Mean minimum	6.40	33.30	32.90	41.30	42.90	47.20	55.50	56.64	48.90	37.90	30.76	22.09	37.98
Mean	13.50	38.50	41.40	49.85	52.70	58.15	68.90	69.56	59.20	46.20	36.88	26.60	46.70
Mean maximum	20.60	43.60	49.90	58.40	62.50	69.10	82.40	82.48	69.60	52.40	43.00	31.19	55.43
1931													
Mean minimum	28.31	27.50	30.58	36.00	45.16	50.50	54.61	55.09	47.76	39.30	26.60	24.87	38.86
Mean	32.75	32.66	37.39	45.48	56.32	60.33	69.59	68.78	57.98	48.87	32.05	29.83	47.68
Mean maximum	37.19	37.82	44.25	54.96	67.49	70.16	84.58	82.48	68.20	58.45	37.50	34.80	56.49
1932													
Mean minimum	22.80	22.90	32.00	38.80	43.64	51.30	53.40	53.40	46.40	40.20	36.20	19.60	38.39
Mean	27.45	28.00	38.24	46.70	53.77	53.05	66.10	66.55	59.95	49.25	42.30	25.75	47.26
Mean maximum	32.10	33.10	44.48	54.70	63.90	74.80	78.80	79.70	73.50	58.30	48.40	31.90	56.14
1933													
Mean minimum	25.11	16.25	31.87	36.80	39.68	49.53	54.06	54.58	43.93	43.29	33.13	33.19	38.45
Mean	30.11	22.59	38.56	46.68	50.37	62.00	69.12	69.13	55.98	53.00	41.15	39.56	48.23
Mean maximum	35.11	28.93	45.26	56.57	61.06	74.47	84.19	85.68	68.03	63.71	49.17	45.93	58.01
1934													
Mean minimum	33.22	34.07	37.58	43.90	47.09	51.47	55.09	54.09	45.40	44.06	37.80	28.16	42.66
Mean	39.46	42.41	47.76	55.55	59.57	62.07	68.17	68.24	56.16	50.83	43.38	32.56	52.18
Mean maximum	45.71	50.75	57.94	67.20	72.06	72.67	81.26	82.39	66.95	57.61	48.97	36.97	61.70
5-year average:													
Mean minimum	23.17	26.80	32.99	39.36	43.69	50.00	54.53	54.76	46.48	40.95	32.90	25.58	39.27
Mean	28.65	32.83	40.67	48.85	54.55	61.12	68.38	68.45	57.85	49.63	39.15	30.86	48.42
Mean maximum	34.14	38.84	48.37	58.37	65.40	72.24	82.25	82.15	69.25	58.09	45.41	36.16	57.56

The heavy precipitation during the winter of 1933–34 had little detrimental effect on the mature grass as far as chemical composition was concerned. There was a slight decline in nitrogen-free extract in the latter part of the winter, but the similar decline in crude fiber and the increase in crude fat would indicate that the small amount of new grass included was largely responsible.

Although the yearly variation in composition at any one date during the growing and maturing season over the 4-year period was slight, particularly when the stage of growth was about the same, the variation in each of these years from the spring flush of new

growth to maturity was very great. Of all nutrients, crude protein showed this variation most decidedly.

As would be expected, the content of crude fiber varied almost inversely with that of crude protein, but its seasonal change was not so decided. The new-growth samples were quite low in crude fiber, exhibiting an increase during maturation, but very little change after maturation. The highest percentage of crude fiber was found in the mature grass collected early in 1932 and 1934.

The precipitation in March and April of 1931 was high but there was none in July and August of that year, and there was little rainfall in May. Yields and test weights of grain grown on the college farm were very low in 1931, indicating rather poor growing conditions. The period from March to June 1933, which includes most of the growing season, was uniformly low in precipitation.

In both 1931 and 1933 there seemed to be a slightly lower percentage of leaves than in the other years as indicated by a higher percentage of crude fiber in the grass collected in these years. The maximum range in crude-fiber content was from 21.23 percent in the new-growth sample collected on April 14, 1933, to 37.84 percent in the old-growth sample collected on February 19, 1932.

According to Woodman and his coworkers (*23*) the process of lignification (production of lignocellulose) is completed at maturity. As practically all of the seeds were shattered out at this time, subsequent changes would be largely owing to the effect of weather. Bluebunch fescue is apparently quite high in the lignocelluloses as it is rather tough and unpalatable during the summer when it is dry but softens in the fall and becomes fairly palatable.

There was not a very definite trend in the content of nitrogen-free extract of the grass samples analyzed largely because of the dissimilarity of the trends of the other nutrients. Undoubtedly there was a high percentage of highly digestible sugars in the new growth as shown by digestion studies on similar grasses (*11*). A larger percentage of the nitrogen-free extract was probably composed of more complex and less digestible compounds as the grass matured (*11*).

The high period in content of nitrogen-free extract came in the summer and fall of 1931. On September 18, 1931, 50.94 percent of the sample was nitrogen-free extract. The first sample of new growth in 1932 taken on April 1 contained only 37.41 percent, which was the low point for the 4-year period. The late summer and early fall of each year was a period in which the nitrogen-free extract was high. At this period the grass was dry, and there was practically no green grass. Toward the end of the winter there was a consistent, though not marked, drop in percentage of this nutrient. Until the bluebunch fescue matured, the decline in percentage of crude protein and crude fat more than counterbalanced the increase in crude fiber and ash resulting in a percentage increase in nitrogen-free extract. After the seeds had shattered and vegetative activity ceased, there followed a gradual decline in nitrogen-free extract. The shattering of seed usually commenced about the middle of July.

The seasonal trend in crude fat was not very regular. The new-growth samples were always fairly high in this nutrient. After a slight diminution in the crude fat of the succeeding few samples, the variations were quite inconsistent. The grass collected in the winter of 1931–32 was lowest in this nutrient. During the late summer

and early fall of 1932 and 1934 there was an unusually high crude-fat content. The weather was not unusual during these periods, but previous growing conditions had been favorable.

The maximum variation in crude fat was from 4.43 percent in the sample for October 28, 1932, to 1.38 percent in the sample taken on September 1, 1933.

SEASONAL VARIATION IN INORGANIC NUTRIENTS

The ash content of all these samples was quite high because it included the silica (table 1). The variation in amount would probably have been somewhat different had the ash been silica-free as the important minerals, calcium and phosphorus, decreased rather than increased with maturity.

The dip in the ash cycle came in the new-growth samples. Although there is more ash in leaves than in stems of grasses, first growth is very tender and consists largely of water and organic matter. The increase in ash during the fall and winter exhibited a rather distinct trend with a few variations, usually reaching a peak the latter part of the winter.

Samples taken during the fall and winter of 1930–31 were all high in ash, reaching a high point of the entire study on February 20, 1931. Crude fiber was lower than usual in the grass collected in this period. This indicates a high percent of leaves high in minerals and low in crude fiber.

Importance attaches to the seasonal variation in calcium because of its relation to the proper nutrition of farm animals. The first growth of the season was richest in this nutrient, with the exception of the 1934 season. In the other 3 years there was a fairly definite downward trend from the first sample of green grass until winter, followed by some recovery late in the winter.

About the most prolonged seasonal decline in percentage of calcium occurred in the bluebunch fescue grown in 1933. Possibly the exceptionally heavy precipitation in December of that year was responsible for the continued decline. The high percentage of calcium in the sample for July 23 of that year was probably because of an accumulation of dust on the grass at the time as the season was quite dry and dusty.

The trend in percentage of calcium was rather unusual in the 1934 season. The first new growth sample taken was rather low in calcium as compared with that collected in the other years. There was an increase rather than the normal decrease in total moisture in the first few samples of new grass, indicating a gradual build-up to the growth peak. There was also an increase rather than a decrease in calcium during this period.

The 1931 and 1933 bunch-grass samples were low in calcium as compared with those of 1932 and 1934. The proportion of stems, which are lower in calcium than leaves, appeared to be higher than usual in 1931 and 1933.

Analyses of semimonthly samples of bluebunch fescue indicate a definite decline in phosphorus, considerably more so than in calcium. The young grass was definitely high for the year in phosphorus. This high point was followed consistently by a rather sharp decline to maturity and then a very gradual decline until winter, when in some cases there was a slight increase for the remainder of the year. Again,

stage of maturity, rather than the effect of weathering seemed to be the principal reason for the decline.

The trend in percentage of phosphorus in these samples very closely followed the trend of crude protein. Both were high in the young grass, folowed by a rather rapid decline until about the time the grass matured. After the grass matured, its content of crude protein leveled off, followed by a small increase during the fall and winter months. The percentage of phosphorus exhibited this trend in most cases, but to a lesser extent.

Undoubtedly the shattering of the matured seeds which are high in phosphorus hastened the decline, but a large part of the total decline had taken place before shattering occurred.

Although stage of maturity was the principal reason for the seasonal decline in phosphorus, there was a greater loss of this nutrient than of calcium after the grass matured. Probably the later decline was largely because of the effects of the weather. No doubt the use of mineral supplements rich in phosphorus would be most effective in alleviating mineral defficiency which might result from feeding bluebunch fescue during the late fall and winter period.

An unusually high percent of phosphorus was recorded for the new growth taken on April 1, 1932, but the sample collected 2 weeks later contained less than one-half as much. The April 1 sample also contained the highest recorded percent of calcium, but the decline in the next sample, collected on April 15, was relatively small compared with that of phosphorus. Apparently the first sample of new growth collected on April 1, 1932, was a little more immature than the first new-growth samples of other years, and the unusually heavy precipitation of the previous month probably caused a mushroomlike growth. The crude protein content for this sample was 25.55 percent, the highest recorded in the 4 years of the study.

In the unusually early season of 1934 the decline in phosphorus was somewhat more gradual than in the previous years. The period of maturation was longer in 1934 than in the other years of this study, and it was during this time that the principal decline in the percentage of phosphorus occurred.

CALCIUM-PHOSPHORUS RATIO

The ratio of calcium to phosphorus is not as important as the amounts of these minerals, but it is often the cause of wastage of the mineral present in larger quantities to conform to the smaller amount of the other.

The ratio of calcium to phosphorus in the skeleton of sheep is 1.30 to 1.00 (*23*), and about the same proportion in feed will best produce that ratio in the skeleton.

Table 1 shows that the early-spring growth was slightly richer in phosphorus than calcium, but at all other times the reverse was true. No extremely wide ratios were found, but the ratio of calcium to phosphorus for the sample taken on February 19, 1932, was 1.00 to 0.27. The highest proportion of phosphorus was contained in the new-growth sample for April 1, 1932, when the calcium-phosphorus ratio was 1.00 to 1.33.

FOUR-YEAR AVERAGE COMPOSITION

Table 4 shows an average of the water-free composition of the samples of bluebunch fescue taken over a 4-year period. Owing to the fact that samples were taken every 2 weeks instead of every 15 days, averages for the middle of the month were periodically included.

TABLE 4.—*Four-year averages of semimonthly analyses of bluebunch fescue*

[Water-free basis]

Period	Ash	Crude protein (N×6.25)	Carbohydrates		Crude fat	Calcium	Phosphorus	Ca to P ratio (Ca as 1)
			Crude fiber	N-free extract				
Mature:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
First part of January	16.22	4.74	32.58	43.55	2.91	0.29	0.14	0.48
Last part of January	16.40	4.73	32.72	43.17	2.98	.30	.13	.43
First part of February	15.26	4.82	33.56	44.07	2.29	.29	.12	.41
Last part of February	16.56	4.90	32.84	43.04	2.66	.30	.14	.47
First part of March	18.82	5.11	31.11	42.33	2.63	.33	.13	.39
Last part of March	18.49	4.99	31.94	42.38	2.20	.31	.14	.45
First part of April	18.58	5.13	30.83	42.52	2.95	.34	.13	.38
New growth:								
First part of March	9.96	17.40	25.96	42.38	4.30	.38	.44	1.16
Last part of March	9.88	18.96	24.08	42.96	4.14	.40	.47	1.18
First part of April	10.36	18.92	25.06	41.96	3.70	.41	.48	1.17
Middle part of April	11.12	16.20	24.01	44.75	3.92	.40	.38	.95
Last of April–first of May	13.20	13.76	28.32	41.16	3.56	.40	.37	.92
Middle part of May	12.91	11.14	28.20	44.27	3.48	.39	.34	.87
Last part of May	12.13	9.21	30.20	45.24	3.24	.36	.28	.78
First part of June	13.09	7.80	31.22	44.51	3.38	.36	.23	.64
Last part of June	13.24	6.54	30.25	46.70	3.26	.37	.22	.59
First part of July	14.01	5.04	31.63	46.27	3.05	.35	.19	.54
Last part of July	13.68	4.62	31.37	46.84	3.49	.36	.20	.56
First part of August	13.67	4.07	31.21	47.68	3.37	.34	.17	.50
Last part of August	14.34	4.13	30.88	47.08	3.57	.37	.18	.49
First part of September	14.56	3.93	28.96	48.74	3.81	.36	.18	.50
Middle part of September	13.58	3.67	30.49	48.85	3.41	.30	.14	.47
Last of September–first of October	15.44	4.18	30.66	46.18	3.54	.32	.14	.44
Middle part of October	16.96	4.60	29.00	45.92	3.52	.32	.15	.47
Last part of October	16.37	4.66	30.28	44.96	3.73	.33	.15	.45
Middle part of November	16.31	4.41	31.63	44.40	3.25	.32	.14	.44
Last of November–first of December	14.82	4.21	32.94	45.16	2.87	.29	.14	.48
Middle part of December	15.45	4.43	32.68	44.39	3.05	.30	.12	.40
Last part of December	17.82	4.64	30.92	43.77	2.85	.31	.12	.39

The percent of crude protein exhibited a regular decline to maturity on this basis and then remained quite constant although there was a slight rise during the winter. The upward trend in crude fiber from the low point in the new grass was not very regular, but the samples taken during the winter period contained the largest amount. The reverse was true of crude fat, which averaged high in the green grass with a rather irregular decline, but a definitely lower average, from November on through the winter. The average content of nitrogen-free extract was low in the new grass and high during the dry period of summer and early fall followed by a decline in the winter months.

The increase in percent of ash from the new growth on through the year was fairly regular. The phosphorus content declined quite regularly through the first part of the winter, leveling off somewhat in the late winter. However, the percent of calcium did not show a very regular or decided downward trend but was highest in the new growth of March and April.

TABLE 5.—*Average composition of semimonthly samples of bluebunch fescue for a 4-year period, by seasons, with standard deviations* [1]

(Water-free basis)

Season	Ash	Crude protein	Carbohydrates		Crude fat	Calcium	Phosphorus	Ca to P ratio (Ca as 1)
			Crude fiber	N-free extract				
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
New growth	12. 12±1. 97	12. 36±4. 88	27. 94±3. 22	44. 02±2. 76	3. 57±0. 49	0. 38±0. 05	0. 34±0. 10	0. 89±0. 22
Maturing	14. 02±2. 35	4. 38± . 87	30. 91±2. 44	47. 25±1. 79	3. 44± . 61	. 36± . 08	. 18± . 03	. 50± . 08
Fall	15. 98±2. 44	4. 41± . 52	30. 90±2. 15	45. 32±1. 74	3. 38± . 65	. 32± . 07	. 14± . 02	. 44± . 12
Winter	16. 78±2. 76	4. 82± . 63	32. 36±1. 99	43. 34±1. 46	2. 70± . 67	. 30± . 04	. 13± . 03	. 43± . 14

[1] $\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$ when n is less than 25; $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$ when n is 25 or more.

SEASONAL AVERAGE COMPOSITION

The analyses from table 1 were averaged by seasons and recorded in table 5. The new-growth season included all new-growth samples to the latter part of June; the maturing season included up to the middle of September; from then to the last of November was considered the fall season; and the winter season carried through to the first new-growth samples. The seasons in this grouping were not all the same length but represented more adequately the stages of growth and dormancy.

The seasonal grouping brings out more clearly the trend in the percentage of the various nutrients in the bluebunch fescue samples analyzed. As would be expected, the standard deviations were quite high for the new growth, particularly for the percentage of crude protein because of the decline in the crude-protein content during that period. There was no significant change in the average percent of crude protein during the 3 other periods.

The average ash content increased quite regularly from the new-growth period through the remainder of the year, but the increase was gradual and not highly significant, particularly after maturity. The trend for calcium and phosphorus was directly opposite from that of crude ash, and the phosphorus trend was significant. Because of the greater decline in phosphorus throughout the growing period, the calcium-phosphorus ratio grew wider, particularly between the first two periods.

The seasonal averages for percent of crude fiber show a slight rise from the new-growth period to the maturing period, followed by very little change during the fall period and then a slight rise through the winter. Because of the variations in each period, the difference between each period was not significant, but there was a significant difference between the new growth and the winter period. The averages for percent of crude fat gradually declined, but the only significant drop was from the fall to the winter period. The nitrogen-free extract percentages for the winter and the new-growth periods were lowest, with a fairly significant difference between periods.

SUMMARY AND CONCLUSIONS

Samples of bluebunch fescue (*Festuca idahoensis* Elmer) were gathered at semimonthly intervals from the fall of 1930 to the fall of 1934, and the analyses are reported in this study.

Any interpretation of a seasonal study of chemical composition of range grasses must be tempered by an examination of the weather records. The months of May, July, and August 1931 were hot and dry. The growing season of March to June 1933 was quite low in moisture, and the summer was hot. In each of these years the appearance of the bunches indicated a higher proportion of stems than in the more favorable years of 1930, 1932, and 1934. There was more of a seasonal increase in the percentage of crude fiber in 1931 and 1933. The percent of crude protein was lowest in the fall and winter of 1931.

Of all the factors causing seasonal and year-to-year variations in chemical composition, stage of maturity was the most important. Particularly did it affect the content of crude protein, causing variations from 25.55 percent in the young growing grass to 4.56 percent in mature grass about 4 months later. This indicates a variation from a protein-rich concentrate to a rather poor roughage. For the remainder of the fall and winter, there was little change in the percentage of this nutrient, and in most cases there was a small increase in the winter. This was probably because of a loss in nitrogen-free extract at this period. The inclusion of a slight amount of the tips of young grass, all of which could not be removed from the samples collected in late winter without losing some of the mature leaves, probably was largely responsible for the slight increase in crude protein at this time.

The percentage of crude fat showed no distinct seasonal variation but was usually highest in the first sample of new grass, after which the trend was variable. However, a summary of the analyses by seasons for the 4 years indicates a fairly significant downward trend as the season advanced. The amount of crude fat was moderate at most times as compared with that of many other forages.

The figures for percent of ash exhibited the opposite trend from that of crude fat, being lowest in the new growth. There was a gradual increase in ash content during the year which leveled off to quite a degree at maturity. The ash content was exceptionally high in view of the percentage of calcium and phosphorus, and there was probably a high percent of silica in the ash.

Nitrogen-free extract was rather a variable quantity, but usually the highest point was during the maturing period and before the fall rains became appreciable. The trend was downward on either side of this peak, being, as a general rule, lower in the new grass and in late winter.

The percent of crude fiber showed a more regular trend, being practically opposite that of crude protein, though not to the same degree. The greatest variation was before maturity, after which the trend was quite variable. Bluebunch fescue had a rather high content of crude fiber.

There was considerably more variation in percent of phosphorus than of calcium. In the young grass there was more phosphorus than calcium, but the decline in the former was considerably faster, continuing through the first part of the winter whereas the decline in percent of calcium leveled off more at maturity. The mature grass was rather low in both, particularly phosphorus. Undoubtedly the shattering of seeds at maturity was partially responsible for the decline in percent of phosphorus, but there was a decided decline in this nutrient before shattering began, which continued after shattering was completed.

As would be expected, the calcium-phosphorus ratio continually widened from the first samples of new growth on through the year. The wide calcium-phosphorus ratio in the mature grass and the small amount of both nutrients would indicate that a mineral supplement containing some calcium and a high percent of phosphorus would be desirable when the feed is largely limited to dry mature grass.

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THE DEVELOPMENT OF MYCOSPHAERELLA BERKELEYII[1]

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SCOPE OF THE WORK

In a previous paper[3] the writer described *Mycosphaerella berkeleyii* as a new species, outlined its life cycle, discussed the symptoms of the disease it produces on peanut (*Arachis hypogaea* L.) and its growth in artificial culture. The present paper is concerned with the cytological details of the development of the spermogonia and perithecia, which have not heretofore been reported.

SPERMOGONIA

As in the case of *Mycosphaerella arachidicola*, the spermogonia of *M. berkeleyii* begin to appear on diseased leaflets during the latter part of September or early in October and continue to develop throughout the fall and early winter. They develop in either leaf surface, but are more often hypophyllous. They originate within the bases of conidiophore stromata, in which case they are well concealed, or in separate stromata that develop after the death of the leaflets. In the latter case they are fairly conspicuous as a zone about the periphery of the lesions on the lower surface of the leaflets, while on the upper surface they may be found scattered throughout the lesion.

The fundaments of the spermogonia arise either subcuticularly or subepidermally. When they arise within conidiophore bases, they are surrounded from the beginning by a thick mass of stroma; but those originating independently of conidiophore bases consist first of loose wefts of interlacing hyphae which continue to grow more complex until a characteristic shape and size have been attained. In the latter case, the thick walls appear stromatous only after the developing spermogonium is exposed through rupture of the cuticle or epidermis.

As development progresses, cells toward the center of the young spermogonium enlarge (pl. 1, *A*), the nucleus of each cell undergoes one or two divisions and ultimately two to four uninucleate spermatia are formed in each cell. The spermatia often lie in tetrads but may appear in linear arrangement within the mother cells, and are liberated through a long sterigma on each mother cell (pl. 1, *B*). The details of spermatial discharge do not differ from that described for *Mycosphaerella arachidicola*,[4] but the thick stromatic wall and the long sterigmata (pl. 1, *B*) are distinguishing features of the spermogonia of *M. berkeleyii*.

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[2] The writer wishes to acknowledge the many helpful suggestions of Dr. B. B. Higgins of this station during the course of the work.

[3] JENKINS, WILBERT A. TWO FUNGI CAUSING LEAF SPOT OF PEANUT. Jour. Agr. Research 56: 317-332, illus. 1935.

[4] JENKINS, WILBERT A. See footnote 3.

PERITHECIA

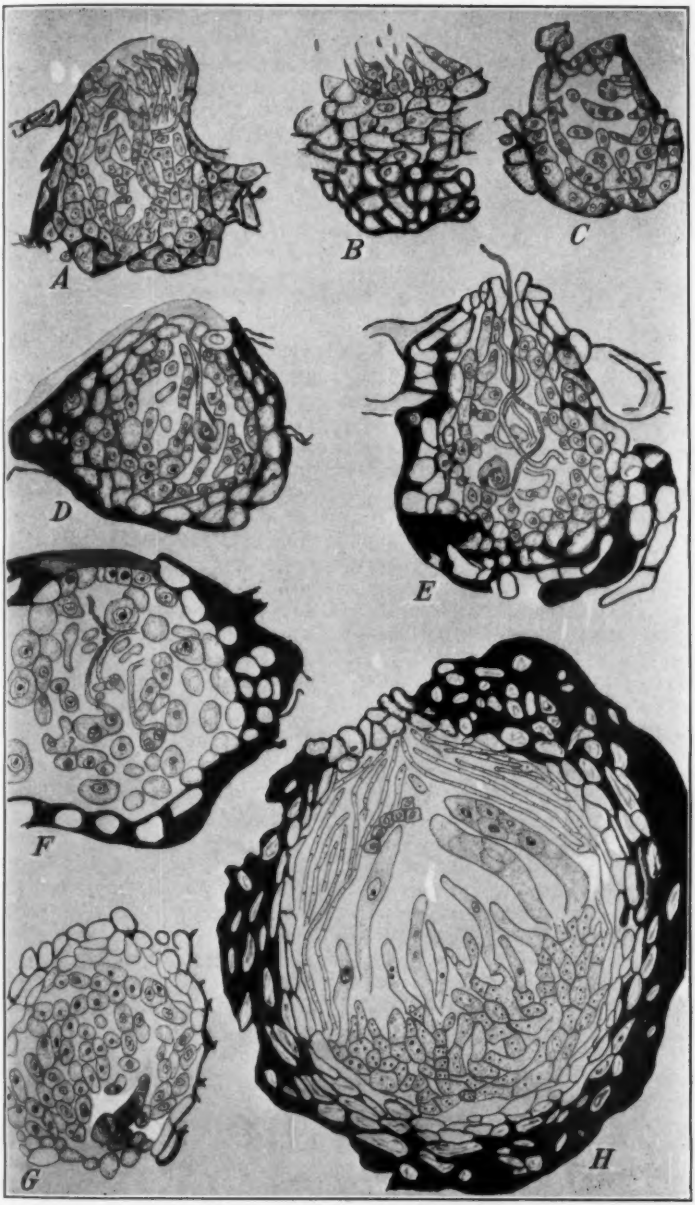
Perithecial fundaments begin to develop during the same period and in similar locations in the leaflets as do the spermogonia. Often they develop close together with only a thin wall separating them. In contrast to the situation in the young spermogonium, the cells toward the center of the young perithecium undergo very little enlargement. Instead, several to many deeply staining archicarps, each with an enlarged, uninucleate basal cell and a long, several-celled trichogyne, are produced. The writer was able to distinguish as many as eight perfectly formed archicarps in a single section of a young perithecium. In the youngest stage seen (pl. 1, *C*), the young archicarps are composed of but two to three cells and each of the cells is from one to four nucleate. Even such early stages show an enlarged basal cell which will ultimately constitute the fertile portion of the archicarp. At functional maturity, the basal cell of the archicarp is uninucleate, as are also the several cells of the trichogyne (pl. 1, *D*). In every case seen, the nucleus within the basal cell of a mature archicarp is considerably larger than the nuclei of the trichogyne, stains more deeply, and has a more conspicuous nucleolus. Owing to the large number of archicarps usually found in each perithecial fundament and the intricate manner in which they coil about the sterile hyphae, it is extremely difficult to obtain sections showing complete archicarps within the fundament. There is, likewise, the problem of obtaining the exterior portion of the trichogyne in its entirety. The exterior parts of the trichogynes of this fungus are quite long. Since they do not coil about in a limited area as do those of *Mycosphaerella arachidicola*, none of the writer's sections showed complete ones.

SPERMATIZATION

Since the exterior portions of the trichogynes were consistently cut off and washed away during the process of preparation of the slides or else remained in such obscure positions that an interpretation of their relationships was hazardous, the writer was unable to follow the initial stages of spermatization. There is much evidence, nevertheless, to indicate that spermatization is not merely a passive phenomenon depending altogether on chance. While free water appears to be essential to the discharge and dissemination of spermatia, the process of spermatization is further assured by the extensive elongation of the exterior portions of the trichogynes which, with a more refined technique, might be shown to actually grow into the vicinity of active spermogonia, even when their origins are relatively remote. Though, as stated above, the earlier stages of spermatization were not seen, it is

EXPLANATORY LEGEND FOR PLATE 1

A, young spermogonium of *Mycosphaerella berkeleyii* in lower surface of leaflet, showing enlargement of cells near center; *B*, portion of mature spermogonium showing origin of spermatia and thick stromatic wall; *C*, young perithecium showing origin of the archicarp. Note the several nuclei in the basal cells and trichogynes of the young archicarps; *D*, young perithecium in which one archicarp has been spermatized, while the other is not yet functionally mature; *E*, young perithecium showing several archicarps, one of which has been spermatized; *F*, portion of a young perithecium showing sperm nuclei within the basal cells of the two archicarps; *G*, portion of an old perithecium in which the one (or more) ascogonia are beginning to branch. Paired nuclei are embedded in dense masses of cytoplasm; *H*, almost mature perithecium (fixed 6–22–37) showing ascogenous hyphae, young asci, almost mature asci and periphyses. All sketches drawn to scale with the aid of a camera lucida. × 1,320.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

assumed that the process is like those which have previously been described.[5] The later stages show no important differences. As the sperm nucleus migrates down the trichogyne, the protoplasm and cross septa disintegrate progressively (pl. 1, *E*). Likewise, the sperm nucleus gradually enlarges during its passage down the trichogyne so that, shortly after it enters the basal cell, it is essentially as large as the basal nucelus (pl. 1, *F*); and a bit later the two nuclei become indistinguishable. Meanwhile, the portions of the trichogyne above the basal cell break down completely (pl. 1, *E*, *F*). No fusion takes place between the sperm and basal nuclei. Instead, the basal cell, or ascogonium, begins to enlarge and as it does so the nuclei divide conjugately.

MATURATION

Following spermatization the apertures at the apex of the young perithecia through which the trichogynes protruded become sealed. This feature, combined with the fact that the stromatic walls are almost impervious to the best cytological fixatives, made it extremely difficult to secure good cytological preparations of the interval between the arrival of the sperm nucleus in the ascogonium and the formation of ascogenous hyphae. Several good preparations were obtained, however, and these show that several pairs of nuclei are formed in the enlarged ascogonium before ascogenous hyphae become evident. Prior to and during the time ascogenous hyphae are being formed, the ascogonium becomes variously branched (pl. 1, *G*). During this period the nuclei continue to divide conjugately, or at least the pairs of nuclei increase in number, and the best preparations show the individual pairs of nuclei embedded in slightly denser masses of cytoplasm than is evident elsewhere in the cells (pl. 1, *G*). The writer could not be certain whether one or more than one ascogonium takes part in the development of ascogenous hyphae within a given perithecium. It is a reasonable assumption, since several archicarps within a young perithecium may be spermatized (pl. 1, *F*), that the fertile portions of the perithecia may consistently arise from several ascogonia.

By the time ascogenous hyphae become evident, the ascogonium, or ascogonia as the case might be, have undergone extensive branching and now cover the floor of the perithecium with a plectenchymatous layer. The asci arise directly or by way of croziers (pl. 1, *H*). The nuclei are usually in pairs and still embedded in dense cytoplasmic masses (pl. 1, *H*). As in the case of *Mycosphaerella arachidicola*,[5] not all the asci mature at the same time, so that ascospore discharge from a given perithecium extends over a considerable period of time.

Concurrent with the development and elongation of the asci, unequal growth stresses arise in the perithecium so that it enlarges and ultimately cracks open at the exposed apical end. During this period certain cells along the wall near the apex of the perithecium become active and form periphyses. Other sterile filaments arise down near the base of the perithecium (pl. 1, *H*). It is uncertain in some cases whether they arise from the fertile layer at the base or from the wall tissue in this area. In any event, no sterile filaments were ever found interspersed among the asci, so that the writer prefers to call them periphyses.

[5] JENKINS, WILBERT A. See footnote 3.

Because of the scarcity of properly fixed material, no great effort was made to trace the cytological development of ascospores. From the best of the preparations studied, ascospore development differed in no important details from that described for *Mycosphaerella arachidicola*.[6]

DISCUSSION

While the general plan of development of the spermogonia and perithecia of *Mycosphaerella berkeleyii* differs in no important details from that of *M. arachidicola*, there are numerous differences in details of structure and organization that have justified this study. These differences taken together with those of spore measurements, time of discharge of ascospores, type of conidial stages, host relationships, and symptoms produced on their mutual host, abundantly justify the erection of separate species for the two fungi.

The consistency of structure in the formation of spermatia as well as the origin and development of the archicarps have been several times illustrated and emphasized in the work of Higgins and the writer,[6] as in the present work. It is not a premature assumption that work of this nature will ultimately lead to a more effective control of these and other similar fungi through a modification of their environment during certain critical stages in sexual reproduction. It is certainly true that this and other work of a similar nature will enable us to better interpret the possibilities of wide variation both in structure and pathogenicity of these fungi through the medium of hybridization.

SUMMARY

The details of structure and development of the spermogonia and perithecia of *Mycosphaerella berkeleyii* differ from those of *M. arachidicola*. The principal differences are: A greater amount of stroma surrounding the fruit bodies, longer sterigmata on the spermatial mother cells, a greater number of archicarps in the young perithecia, a greater amount of fertile tissue in the base of the perithecium, origin of periphyses, and the tardiness of ascospore discharge in *M. berkeleyii*. In addition, as stated in a previous paper, the two fungi produce different symptoms on their mutual host, differ in type of conidial fructifications, host relationships, culture characteristics, and size of ascospores.

[6] JENKINS, WILBERT A. See footnote 3.

UNFRUITFULNESS IN THE TOMATO CAUSED BY MALE STERILITY[1]

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INTRODUCTION

Some apparently healthy but unfruitful individuals usually occur in tomato fields on the Pacific coast. As the crop matures, these unfruitful plants are often conspicuous for their vegetative vigor and upright habit. Since the unfruitful plants are distributed apparently at random among the normal plants, many of them appearing healthy, disease or unfavorable soil or climatic conditions can hardly be the cause. The unfruitfulness is usually partial and often affects only a fraction of 1 percent of the plants.

ORIGIN AND CAUSE OF UNFRUITFULNESS

At Riverside, Calif., in 1933 in an F_3 population consisting of 68 plants, 1 apparently healthy but highly unfruitful plant occurred. The P_1 plants were a triploid (female) and a diploid (male), and F_1 was a trisomic having an extra H (IV) chromosome and probably another extra chromosome that was not identified. The F_1 parent was a simple trisomic, triplo-H, homozygous for the gene d_1 (dwarf), and heterozygous for *c* (potato leaf). Incidentally, inheritance of *c* in F_2 and F_3 was trisomic, showing that the locus of *c* is in chromosome IV (*H*). The F_3 population was generally healthy, matured its fruit very late in the season, and tended to be unproductive. The triplo-*H* plants were as usual especially unfruitful, but one apparently diploid plant also had this characteristic. In another F_3 family from the same F_2 parent planted the following season (1934), the diploid progeny consisted of 115 relatively fruitful plants and 8 unfruitful plants similar to the single unfruitful diploid previously observed. At the same time 2 F_3 progenies of other F_2 plants, consisting of 44 and 28 plants, contained no unfruitful diploids. In 3 F_4 populations from fruitful F_3 sibs of the original unfruitful plant, no unfruitfuls occurred, but in the fourth there were 40 fruitfuls and 10 unfruitfuls. Severe nematode infection in this season (1934) made classification uncertain, but some of these unfruitfuls seemed healthy and free from nematode symptoms.

Unfruitful diploid plants from these and related populations were self-pollinated in the greenhouse where conditions were favorable for fruit setting, but no fruit set. They readily functioned as seed parents and no difficulty was found in obtaining fruit and seeds when pollen of fruitfuls was applied.

Other unfruitful plants at Riverside and San Jose, Calif., proved to be triploid or rarely simple trisomic or tetraploid. The unfruitful plants discussed in this paper did not resemble any known chromosome mutant. Although their origin from a double trisomic one extra

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chromosome of which was not identified and the general unfruitfulness of trisomics suggested some visible chromosomal irregularity, these unfruitful plants proved to have the normal diploid chromosome number and distribution.

The nonabortion of the anthers and the nearly normal amount of pollen were not especially suggestive of a male-sterile gene mutant. However, germination tests were made with mature pollen of fruitfuls and unfruitfuls in van Tieghem cells. In 15-percent cane sugar alone practically no germination occurred, but with two or three stigmas added, as a rule about 10 to 25 percent of the grains of normal plants germinated in a few hours. Only from 0 to about 0.5 percent of the pollen grains of unfruitful plants germinated, and pollen-tube growth was much slower.

POLLEN CHARACTERISTICS.

In acetocarmine smears, mature pollen of fruitful and unfruitful plants looks much alike, although the pollen of unfruitful plants is somewhat smaller and more alveolar than that of fruitful. In a dilute solution of iodine in potassium iodide, however, the two kinds of pollen were very different. Pollen smears from *mature* flowers of fruitful plants consist of numerous large, clear, pale-yellow grains, some smaller alveolar grains, and some empty grains. In diseased plants, or after very hot weather in healthy plants, the number of empty grains is greatly increased. The latter are indicative of early abortion of pollen. Anthers from open flowers of unfruitful plants very often look more waxy and paler yellow than mature anthers of fruitfuls. Pollen from the anthers of fully opened flowers of unfruitfuls, when stained in iodine, shows that most of the pollen cells contain starch (fig. 1). A very few grains have no starch; many contain a great many large black starch grains. Smears from mature anthers of unfruitfuls appear grayish to the naked eye; those of fruitfuls a clear yellow. If pollen from slightly unripe anthers of fruitfuls is stained with iodine, the smear is like that from mature flowers of unfruitfuls. In other words, normally the starch in young pollen changes by hydrolysis, presumably to dextrins, as the pollen matures. This process is, in some way, arrested in the unfruitful plant. This arrested development has been found to be typical of unfruitfuls at all seasons. Pollen of F_1 hybrids between unfruitful (female) and fruitful (male) cannot be positively distinguished from that of the fruitful parent. Evidently the unfruitfulness is due to a form of male sterility in which pollen development is arrested and almost all the pollen is nonviable.

The most obvious characteristic of the male-sterile type is unfruitfulness (fig. 2). It is possible that other differences might be found in populations that are otherwise more nearly homozygous. Unfruitfulness is seldom, if ever, total. In the progeny of C337–1, with fruits from open pollination, the average weight of 8 fruits from a male-fertile plant was 41 gm., and of 12 fruits from three male-sterile plants, 28 gm. The corresponding average number of seeds was 59 and 24, respectively. In the progeny of C324–2, the average weight of 55 fruits from three male fertiles was 26 gm., and of 8 fruits from two male steriles, 19 gm. The average number of seeds was 36 and 19, respectively. Accordingly the fruit weight of the male fertiles

was about 50 percent greater and the number of seeds about double that of the male steriles.

In some progenies, the male steriles can be picked out with certainty by their outstanding unfruitfulness; but in others, owing to grada-

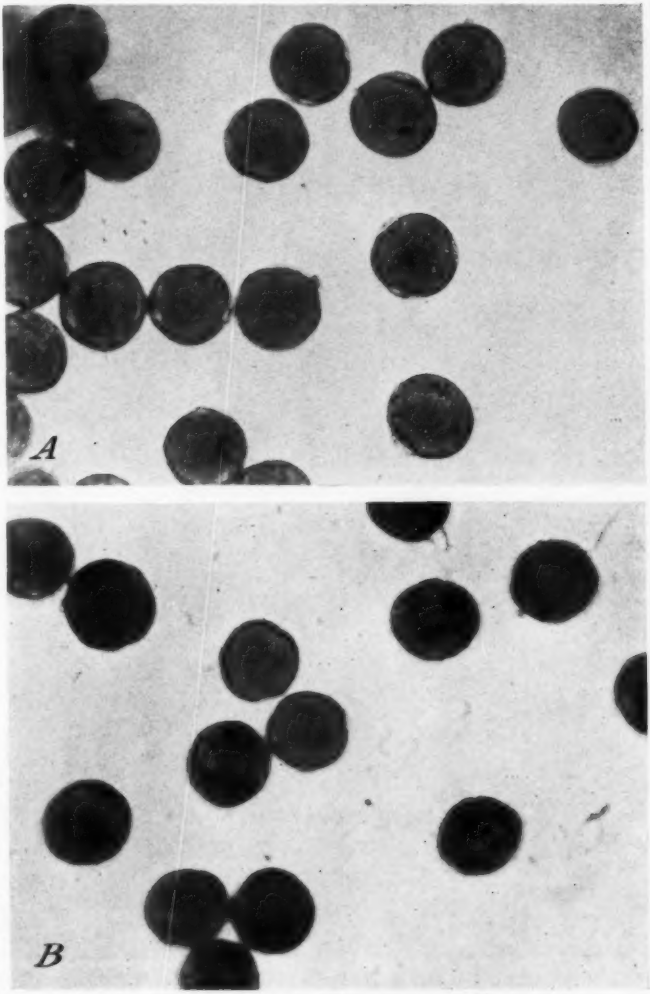

FIGURE 1.—Male-fertile (*A*) and male-sterile (*B*) tomato pollen stained in aqueous iodine-potassium iodide solution. × 593.

tions in fruitfulness, they can only be distinguished from the less fruitful male-fertile plants by the pollen test. Prior to 1935 the plants were classified only for fruitfulness without applying the pollen test, and some of the unfruitful plants may have been male-fertile. These

earlier data are not included in table 1. In 1935 and 1936, as shown in table 1, the pollen of all plants that were either unfruitful or low in fruitfulness, and in 1937 the pollen of every plant, was tested in iodine; and in doubtful cases three or four pollen examinations were

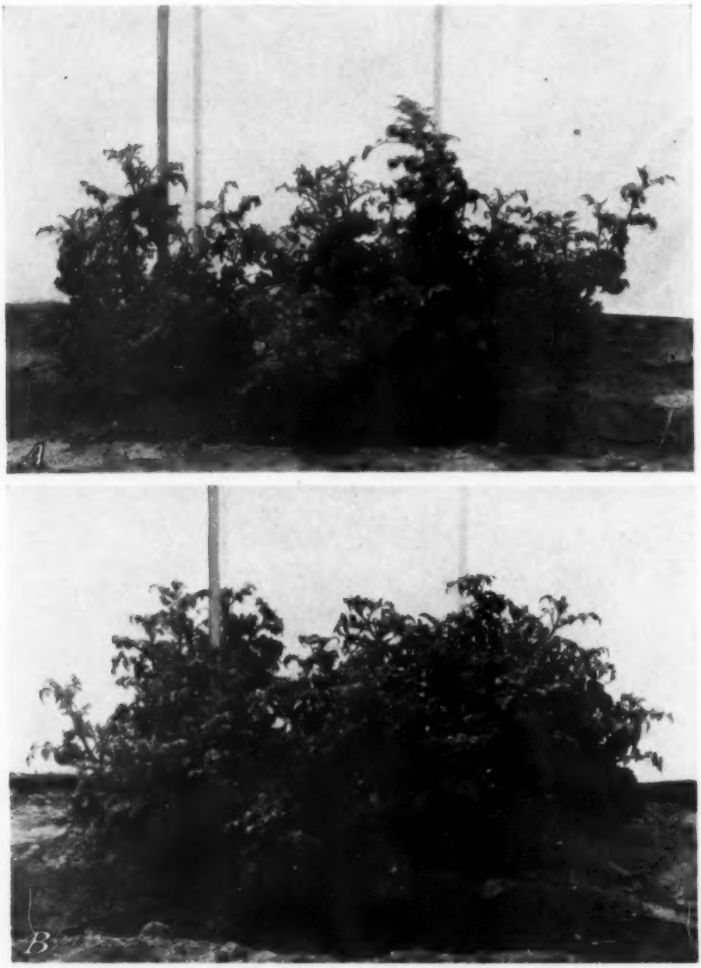

FIGURE 2.—Male-fertile (*A*) and male-sterile (*B*) tomato plants showing difference in fruitfulness. Photographed November 1, 1936.

made. An attempt was made to correlate degrees of fruitfulness with the quality of pollen determined in iodine, but no close correlation was found. In only two cases unfruitful plants proved to have normal pollen. Both of these plants subsequently set fruit readily from selfing in the greenhouse.

TABLE 1.—*F_2 progeny of male sterile × male fertile and backcross progeny of male sterile × (male sterile × male fertile) tomatoes, 1935–37*

Parents	Progeny						
	Observed		Expected [1]			Plants whose pollen was examined by iodine test	Recorded [2]
	Male fertile	Male sterile	Monohybrid	Dihybrid	Trihybrid		
F_1, selfed:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
C307–3	102	16	88.5:29.5	111:7		39	73
C322–2	68	1		65:4	68:1	3	64
C324–1	63	6		65:4		11	32
C324–2	31	2		31:2		33	30
C337–1	175	15		178:12		42	91
C339–1	249	15		247:17		99	87
C328–1	36	3		37:2		39	36
C342–1	59	5		60:4		64	59
C342–2	31	1		30:2	31.5:0.5	32	63
C338–1	93	19	84:28	105:7		112	69
Backcrossed:							
C218–8–1–4–1 × C322–2	31	6		28:9		13	34
C218–8–1–1 × C324–1	5	0		4:1		0	9
C218–8–1–4 × C327–3	118	21		104:35	122:17	91	86
C337–1–2 × C328–1	41	21	31:31	46.5:15.5		62	77
C337–1–2 × C324–3	17	14	15.5:15.5	23:8		31	38
C337–1–7 × C324–3	18	2		15:5		20	91
C337–1–7 × C324–3 (limited pollination)	7	4		8:3		11	79
C337–1–4 × C324–3	22	5		20:7		27	100
C337–1–4 × C324–3 (limited pollination)	16	1		13:4		17	63

[1] Computed as a rule to the nearest whole number.
[2] This column gives the percentage of seeds planted which produced plants that were recorded as to pollen fertility.

Seeds were planted from fruits of male-sterile plants carrying recessive genes such as d_1 (dwarf), *c* (potato leaf), and a_1 (green stem), which grew adjacent to plants carrying the dominant alleles. Evidently any resulting seedling having a character dominant to the corresponding character of the male-sterile parent resulted from cross-pollination, and a seedling not having such a dominant character may have resulted from selfing. Among 152 plants from male-sterile parents, about 28 percent had characters dominant to those of the mother plant. Clearly a large proportion of the seeds resulted from cross-pollination. Although in the greenhouse attempts at self-pollination have always failed, conceivably some self-fertilization might occur if the pollen of male-sterile plants were mixed with a small proportion of fertile pollen.

GENETICS OF MALE STERILITY

All F_1 plants from P_1 male steriles pollinated by five different unrelated male-fertile parents were self-fruitful, and all those tested had normal pollen. Male sterility was completely recessive. The earlier (1933 and 1934) data suggested that male sterility was not a simple recessive. The ratios among the F_2 progeny of male steriles by unrelated male fertiles grown in 1935–37 are shown in table 1. All except the F_2 from C338–1 closely approach the dihybrid ratio, 15:1. The conformity of these F_2 ratios (table 1) to the expectation on a 15:1 basis seemed close enough to justify summation. In all F_2 families, excepting that from C338–1, the ratio of male fertile to male sterile was 814:64; and the expectation on a 15:1 basis is 823:55 ($X^2=1.6$, $P=0.2$). The F_1 and F_2 data therefore indicate that male sterility

probably depends on two recessive genes. Adopting the symbols used in maize, the genes are termed ms_1 and ms_2. Male-sterile plants are homozygous for both these recessive genes, and male-fertile plants contain either one or both of the dominant alleles MS_1 and MS_2.

Four of the five different male-fertile (P_1) parents that were unrelated to the male-sterile plants in these crosses may have been homozygous for one or both of the dominant alleles MS_1 and MS_2. In the F_2 from C339–1, the ratio was 250:15, or very nearly 15:1, but in the F_2 family from C338–1, a plant which originated from another male sterile crossed with the same male fertile 35.020.1, the observed ratio, 93:19, is distinctly nearer 3:1 ($X^2=3.9$, $P=0.05$) than 15:1 ($X^2=22$, $P=0.001$). The occurrence of 3:1 and 15:1 F_2 ratios from the same male-fertile P_1 parent, 35.020.1, suggests that it was heterozygous for at least one of the male-sterility genes although unrelated to the male-sterile line. Twenty F_1 plants of the same parentage as C338–1, grown in the greenhouse, were all male fertile, although the pollen contained more alveolar grains than the typical male fertile. Probably the P_1 plant 35.020.1 was $MS_1ms_1MS_2ms_2$.

Two male-fertile F_1 plants, C324–1 and C322–2, which from selfing gave 63:6 and 68:1 ratios, were backcrossed with male steriles C218–8–1–1 and C218–8–1–4–1, and gave ratios of 5:0 and 31:6, respectively. Two other backcrosses, C337–1–7 × C324–3 and C337–1–4 × C324–3, gave 25:6 and 38:6 ratios. These agree fairly well with the 3:1 ratio expected from a backcross of a double recessive male sterile with a male fertile heterozygous for two pairs of male-sterility genes. In the progeny of the backcross C337–1–2 × C324–3, the observed ratio 17:14 is nearer 1:1 ($X^2=0.29$, $P=0.59$) than 3:1 ($X^2=6.7$, $P=0.01$), but in the other backcrosses mentioned above, with the same male-fertile parent C324–3, the ratios (25:6 and 38:6) were nearer 3:1 than 1:1, so that C324–3 was probably MS_1 ms_1 MS_2 ms_2. In the backcross progeny of C337–1–2 × C328–1, the observed ratio was 41:21, which is nearer 3:1 ($X^2=2.6$, $P=0.1$) than 1:1 ($X^2=6.5$, $P=0.01$). The F_1 male-fertile parent, C328–1, gave in F_2, 36 male-fertile and 3 male-sterile plants, or 12:1, so that it is uncertain whether this F_1 was MS_1 ms_1 ms_2 ms_2 or Ms_1 ms_1 Ms_2 ms_2. The backcross family, C218–8–1–4 × C327–3 contained 118 male-fertile and 21 male-sterile plants, a ratio considerably nearer 7:1 ($X^2=0.86$, $P=0.3$) than 3:1 ($X^2=7.5$, $P=0.006$). This family was very vigorous, and the greater fruitfulness of male-fertile as compared with male-sterile plants was especially conspicuous. Two male-fertile plants C342–1 and C342–2 from this family gave on selfing 59:5 and 31:1 ratios. The 7:1 backcross ratio suggests that the male-fertile parent C327–3 was heterozygous for three pairs of male-sterility genes. This plant itself originated in a backcross of C218–8–1–4–1 and C322–2 that consisted of 31 male-fertile and 6 male-sterile plants. Possibly a third male-sterility gene was introduced in the unrelated male-fertile parent of the F_1 plant C322–2. The total ratio in the backcross progenies, excepting C218–8–1–4 × C327–3, is 157:53 and is almost exactly the expected dihybrid ratio.

The F_2 and backcross data therefore indicate that male sterility is genetic and depends on the presence of two or, in some genotypes, possibly three recessive genes which are either independent or not closely linked.

TABLE 2.—*Relation between male sterility and other genes* [1]

Parents	Derivation	Genes entering F_1 together with male sterility	Progeny: Male fertile or sterile	Chromosome I: *D*	*d*	*P*	*p*	*S*	*s*	Chromosome II: *R*	*r*	Chromosome III: *Y*	*y*	Chromosome IV: *C*	*c*	*Sp*	*sp*	Chromosome V: *A*	*a*	Chromosome VI: *L*	*l*
				No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
C307-3	*S*	*drC*	+	76	26					75	27			79	23						
			O	14	2					11	5			14	2						
C322-2	*S*	*dS*	+	53	15			59	9												
			O	1	0			0	1												
C324-1	*S*	*PSR*	+			43	19	52	12	48	14										
			O			4	2	3	3	3	3										
C324-2	*S*	*PSRYa*	+			25	5	26	4	23	7	19	11					26	7		
			O			2	0	2	0	0	2	2	0					2	0		
C337-1	*S*	*RYCAL*	+							137	43	136	44	128	50			138	41	134	44
			O							9	4	12	1	11	4			13	2	10	5
C339-1	*S*	*dCSp*	+	193	66									178	77	165	80				
			O	13	2									10	5	11	4				
C328-1	*S*	*PSRa*	+			24	11	30	6	23	13							28	8		
			O			3	0	3	0	2	1							1	2		
C342-1	*S*	*a*	+															39	16		
			O															5	0		
C342-2	*S*	*S*	+					31	2												
			O					0	1												
C338-1	*S*	*drCSp*	+	75	16					70	18			70	31	70	32				
			O	15	4					11	7			16	3	15	3				
C218-8-1-4-1×C322-2	*B*	*d*	+	19	12																
			O	2	4																
C218-8-1-4×C327-3	*B*	*a*	+															55	62		
			O															10	12		
C337-1-2×C324-3	*B*	*RY*	+							15	1	7	9								
			O							12	2	9	5								
C337-1-7×C324-3	*B*	*R*	+							10	14										
			O							2	2										
C337-1-4×C324-3	*B*	*R*	+							18	21										
			O							2	3										

[1] The phenotypes are represented by their corresponding gene symbols; + signifies male fertile; *O*, male sterile; *S*, that progeny was derived from selfing; *B*, that it was derived from backcrossing.

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The percentage of seeds that germinated and were eventually recorded as plants with respect to their pollen is shown in the last column of table 1. This percentage was used as a measure of viability; the mean value was determined in families having an excess of male steriles over the expectation and in progenies with a deficiency of male steriles. The mean viability of excess male-sterile families was 64 percent and that of deficiency families 71 percent; the difference is probably not significant. Other evidence that male-sterile plants have normal viability is the fact that no consistent difference in vigor is evident between male-fertile and male-sterile plants in the field.

To test the possible influence of pollen competition, or certation, on the proportion of male-sterile progeny, an amount of pollen was applied which seemed barely sufficient to fertilize all the eggs. A few pollen grains of plants heterozygous for male sterility were picked up on a human hair and applied to the stigma of a male sterile. The average number of seeds in four fruits from limited pollination was 10, as compared with over 50 in seven fruits of the same plants from unlimited pollination. Presumably the amount of pollen was sufficiently limited to prevent certation between pollen carrying male sterility and that carrying male fertility. In one cross there were fewer male steriles from limited pollination, but in the other the relation was reversed. This suggests that pollen certation does not greatly influence the frequency of transmission of male sterility.

Alleles of certain genes present in the male-sterile parents were introduced to test the relation between the male-sterility genes and genes in six different linkage groups (tables 2 and 3).

TABLE 3.—*χ-square test of association between male sterility and other genes in the combined F_2 and backcross (B) progenies* [1]

Generation	Gene entering F_1 together with male sterility	Chromosome No.	Observed phenotypic ratios				X^2	P	
			Male fertile		Male sterile			Positive	Negative
			X	*x*	*X*	*x*			
F_2	d_1	I	397	123	43	8	1.25		0.27
B	d_1	I	19	12	2	4	.65	0.42	
F_2	*P*	I	92	45	9	2	.45	.50	
F_2	*S*	I	198	33	8	5	3.8		.05
F_2	*r*	II	145	45	22	12	1.5	.22	
F_2	*R*	II	231	77	14	10	2.4		.12
B	*R*	II	43	36	16	7	1.2	.27	
F_2	*Y*	III	155	55	14	1	1.9	.17	
B	*Y*	III	7	9	5	5	.58	.45	
F_2	*C*	IV	455	181	51	14	1.1	.29	
F_2	*Sp*	IV	235	112	26	7	1.2	.27	
F_2	a_1	V	93	31	8	2	.00	1.0	
F_2	A_1	V	138	41	13	2	.27	.60	
B	a_1	V	55	62	10	12	.01		.92
F_2	*L*	VI	134	44	10	5	.01		.92

[1] An association, measured by *P*, of male sterility with the gene entering F_1 together with male sterility is termed positive and would be evidence of genetic linkage. An association of male sterility with a gene not entering F_1 together with male sterility is negative. *X* represents the dominant, *x* the recessive phenotype. *P* is the probability that the observed frequencies would occur by chance, in case of independence. *B*=backcrossed.

The χ-square test of association, with Yates' correction, was applied.[2] As is evident from table 3, there was no significant association of male sterility with the chromosome I genes d_1 and *P;* and

[2] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 6, rev. and enl., 339 pp., illus. Edinburgh and London. 1936.

the apparent association with *S* is not due to linkage. There is no evident association of male sterility with *R*, *r*, *Y*, A_1, a_1, or *L* but possibly a suggestion of linkage with the genes *C* and *Sp* in chromosome IV, the odds being about 2.6 to 1 in favor of association. Clearly it is not proved that male sterility is independent of these six linkage groups but only that it is probably not closely linked with any of the genes involved in the tests. For the genetic study of male sterility alone it would probably have been better to introduce as few genes as possible allelic to those in the male-sterile parents.

DISCUSSION

Male sterility in the tomato was reported by Crane.[3] It behaved as a recessive character and was believed to depend on a mutant gene. In maize 20 genetically distinct types of male sterility have been described by Emerson, Beadle, and Fraser,[4] most of them depending on single recessive genes. The dependence of male sterility on at least two genes, as in the present instance, seems to be rather exceptional. If two recessive alleles must be present to produce male sterility, they appear to be complementary genes, and male sterility should occur in F_2 from crossing male-fertile races having different male-sterility genes. Male sterility is not due to gametophytic lethals, since heterozygous plants may be quite male fertile and the backcross male sterile $\times$ (F_1 male sterile $\times$ male fertile) gave in part male-sterile progeny, whereas male sterile $\times$ unrelated male fertile gave only male-fertile progeny. It is due to genes in the sporophyte which cause the development of the microspores to be arrested and which therefore have a lethal effect.

In the tomato, fruitfulness as a rule depends on self-pollination. In the greenhouse, with many varieties hand pollination is necessary. In the field, agitation by wind is usually sufficient to shake onto the stigma the pollen that is shed within the tube formed by the anthers Bumblebees and other insects also assist to a much less extent in pollination. Since self-pollination is the rule, defective pollen is potentially a major cause of unfruitfulness. Unfruitfulness in triploid tomatoes has been shown to be due to this cause. In the present instance, since the amount of pollen produced is nearly normal, some other cause of unfruitfulness might easily be expected. One of the five unrelated male-fertile plants used in crossing is apparently heterozygous for both male-sterility genes. It is by no means improbable that some of the unfruitful plants that occur in commercial varieties are genetically male sterile. Plants heterozygous for two male-sterility genes would give on selfing only one-sixteenth of male-sterile progeny, and owing to the complete recessiveness of male sterility, they would not tend to be eliminated by mass selection. Male sterility depending on two recessive genes could be eliminated from a seed stock by single-plant selection, if a sufficiently large population from selfing of each selected parent were tested. According to Warwick,[5] on a 15:1 basis the odds are 21:1 in favor of the occurrence of at least one recessive in a population of 48 plants.

[3] CRANE, M. B. HEREDITY OF TYPES OF INFLORESCENCE AND FRUITS IN TOMATO. Jour. Genetics 5: 1–11, illus. 1915.

[4] EMERSON, R. A., BEADLE, G. W., and FRASER, A. C. A SUMMARY OF LINKAGE STUDIES IN MAIZE N. Y. (Cornell) Agr. Expt. Sta. Mem. 180, 83 pp. 1935.

[5] WARWICK, B. L. PROBABILITY TABLES FOR MENDELIAN RATIOS WITH SMALL NUMBERS. Tex. Agr. Expt Sta. Bull. 463, 28 pp., illus. 1932. See table 4.

It is not suggested that fruitfulness in tomatoes is determined by only two or even three pairs of genes. Variation in fruitfulness occurred in these cultures which could not be related to male sterility. For example, in certain populations in which segregation of D_1 (standard) and d_1 (dwarf) occurred, the unfruitful condition was less extreme in standard plants, and where segregation of L (normal green) and l (lutescent) occurred, the normal green plants tended to be more fruitful. Evidently other genes affecting fruitfulness occur, some of which might be studied in relation to known genes.

Apparently the mutation that resulted in the formation of a gamete carrying male sterility occurred in a somatic cell or a spore mother cell of the triploid × diploid F_1 plant 30.059 .41, since only one of the three F_2 plants tested proved to be heterozygous for both male-sterility genes. Possibly the fact that the F_1 plant was a double trisomic made conditions favorable for the simultaneous occurrence of two gene mutations, but a more probable hypothesis is that this plant was already heterozygous for one mutant gene and that a second mutation occurred in it.

It is suggested that some of the unfruitful plants that occur in commercial varieties are male-sterile segregates from the selfing of fruitful and apparently normal heterozygous plants.

SUMMARY

Partially unfruitful plants occurred in an F_3 population from a simple trisomic F_2 plant having an extra H (IV) chromosome. The F_1 plant was probably a double trisomic having an extra H (IV) chromosome and another extra chromosome not identified. The pollen produced by the male sterile is nearly normal in amount, but its development is arrested. In mature anthers the pollen is distinguished in aqueous iodine by its slightly smaller size, darker color, and coarser texture and by the prevalence of starch granules in the cytoplasm. A small proportion, perhaps 2 to 3 percent, of fully developed grains occurs, but apparently they do not function. Male sterility is completely recessive and depends on at least two recessive genes, ms_1 and ms_2, both of which are necessary for male sterility. In F_2 from male sterile × male fertile, approximations to 15:1 and 3:1 ratios, and in backcrosses usually 3:1 ratios occurred. An apparent 7:1 ratio in one backcross suggests that the parents may have differed in a third pair of male-sterility genes, the third dominant allele being introduced by the male-fertile parent. One of the five unrelated male-fertile races was heterozygous for one or probably both mutant genes.

THE CALCIUM-PHOSPHORUS RATIO OF THE SKINS OF CANNING PEAS AND ITS RELATION TO MATURITY[1]

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INTRODUCTION

The chemical changes that take place in peas (*Pisum sativum* L.) with maturity have been studied by many investigators (*2, 4, 5, 8, 10, 11, 12, 13*)[3] in an effort to discover and perfect chemical methods for determining the quality of the canned product. Sayre, Willaman, and Kertesz (*11*) found that the calcium content of pea skins increases with maturity, and later, Kertesz (*9*) reported that the alcohol-insoluble solids also increased with maturity and serve as a good index to quality. Thompson and Peterson (*14*), in studying the chemical composition of the whole pea, found that calcium and phosphorus increased with maturity when the percentage was calculated on the wet basis, but remained approximately constant when the percentage of each was calculated on the oven-dry basis. Previous work at the Idaho station (*1*) has shown that the skins of mature peas have a wide calcium-phosphorus ratio.

The work described in the present paper was undertaken to obtain data on the calcium-phosphorus ratio of the raw canning-pea skins at different stages of maturity and to determine, if possible, its relationship to the quality of the canned pea.

MATERIAL AND METHODS

The raw canning peas used for this work were obtained from a commercial cannery at Ellensburg, Wash. When the peas arrived from the viner they were separated according to the standard sieve sizes and the skins were immediately removed from the cotyledons by hand. The skins from the respective sieve sizes were washed, dried, and sent to the laboratory for chemical analysis of calcium and phosphorus. From the same lot of peas, canned samples of the respective sieve sizes were taken for the organoleptic grade and for calcium and phosphorus analyses of the skins. The organoleptic grade was determined by two officials of a commercial cannery, each grading independently of the other, and their results were averaged. Where there is no organoleptic grade indicated, the canned samples were lost in transportation.

Two grams of air-dried pea skins were ashed in a platinum dish at 500° C. The ash was dissolved in 2 ml of 6 N hydrochloric acid and made up to a definite volume of 100 ml with distilled water. An aliquot of this solution, representing 0.1 g of pea skins, was analyzed

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[2] The authors wish to express their appreciation to the Bozeman Canning Co. of Mount Vernon, Wash., for supplying the pea samples.

[3] Italic numbers in parentheses refer to Literature Cited, p. 635.

colorimetrically for phosphorus according to the method of Fiske and Subbarow (*7*).

The calcium was also determined colorimetrically, as described by Emmert (*6*). A 25-ml aliquot, corresponding to 0.5 g of the pea skins, was transferred to a 50-ml volumetric flask, made alkaline to Phenolphthalein with 10-percent sodium hydroxide and 2 ml of 10-percent sodium hydroxide added in excess. The contents were made up to volume and filtered. An aliquot of the alkaline filtrate equivalent to 0.2 g of the pea skins was transferred to a 50-ml volumetric flask, acidified with dilute sulphuric acid, and the phosphorus was determined colorimetrically as the alkaline-soluble phosphorus. Known amounts of phosphorus were added to those canned samples that did not have enough phosphorus present to precipitate all the calcium and did not give a color of sufficiently high intensity for colorimetric reading. The difference in percent between the acid-soluble phosphorus and the alkaline-soluble phosphorus was multiplied by 1.93 to obtain the percentage of calcium.

Although the colorimetric determination of calcium gave slightly higher results than the commonly used oxalate method, it offered a simple and rapid calcium determination with precise comparative results. The numerical calcium-phosphorus ratio, as given in the following tables, was derived as follows:

$$\text{Calcium-phosphorus ratio} = \frac{\text{Percent calcium} \times 100}{\text{Percent phosphorus}}$$

RESULTS AND DISCUSSION

Table 1 shows the variation in percentage of calcium and phosphorus and the calcium-phosphorus ratio of the skins obtained from different sieve sizes of two varieties of raw canning peas. The organoleptic grade is also given for a few samples.

With few exceptions the results in table 1 show an increase of calcium and a decrease of phosphorus in the raw pea skins with advancing stages of maturity, when maturity is based on seive size. The increasing calcium content and the decreasing phosphorus content of the skins result in a wider ratio as the pea matures. The increase in calcium content with maturity is in agreement with the work of Sayre, Willaman, and Kertesz (*12*), who state that there is a migration of calcium to the skin as the pea matures. Thompson and Peterson (*14*) state that no difference was found between the calcium and phosphorus content of the whole pea with maturity when calculated on an oven-dry basis; one may assume, therefore, that there is a migration of calcium from the cotyledon with advancing maturity. Boswell (*3*) reports a decrease in nonprotein nitrogen and an increase in protein nitrogen with maturity. It is possible that the migrating phosphorus from the skins combines with the increased protein nitrogen of the cotyledon as a phosphoprotein.

It is recognized that the size of the pea is not always an index to maturity. Environmental factors, such as prolonged drought, insects, etc., may sometimes prevent the enlargement of the pea without hindering maturation. In spite of these factors, however, the size of peas from the same lot, grown and secured under the same conditions, can be used as a general index to maturity.

TABLE 1.—*Calcium and phosphorus content, calcium-phosphorus ratio, and organoleptic grade of the skins of different sieve sizes of 2 varieties of raw canning peas*

Sample	Variety	Phosphorus in sieve size—			Calcium in sieve size—			Ca/P ratio×100 for sieve size—			Organoleptic grade of sieve size—		
		3	4	5	3	4	5	3	4	5	3	4	5
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*						
B	Perfection	0.376	0.329	0.263	0.215	0.265	0.302	57.2	80.5	114.8			
D		.334	.297	.192	.240	.290	.292	71.9	97.6	152.1			
F		.245	.214	.150	.214	.286	.264	87.3	133.6	176.0	Extra Standard	Extra Standard	Standard.
G		.400	.278	.191	.312	.315	.338	78.0	113.3	177.0	Fancy	do	Do.
A	Lot 100 [1]	.393	.423	.352	.236	.248	.248	60.1	58.6	70.5			
C		.566	.432	.275	.196	.216	.278	34.	50.0	101.1			
E		.222	.198	.149	.194	.263	.254	87.4	132.8	170.5			
H		.258	.178	.168	.294	.322	.321	114.0	180.9	191.1	Extra standard	Standard	Standard.
I		.374	.267	.221	.241	.306	.301	64.4	137.1	136.2	Fancy	Extra standard	Do.
J		.301	.303	.209	.283	.338	.295	94.0	111.6	140.2	Extra standard	do	Do.

[1] A selection from Profusion, developed at the Idaho station.

The above factors must be taken into consideration in interpreting the data in table 1, especially in comparing the calcium-phosphorus ratio of peas grown under different field conditions. For example, in table 1, the calcium-phosphorus ratio of sieve size 4 in sample B is 80.5, the calcium-phosphorus ratio of sieve size 3 in sample F is 87.3, indicating that sieve size 3 of sample F is more mature than sieve size 4 of sample B. This finding is further substantiated by the calcium-phosphorus ratio of sieve sizes 4 and 5 of sample F. In those samples having an organoleptic grade, there exists a fairly high degree of correlation between the calcium-phosphorus ratio and the given grade. In sample H the peas of sieve size 3 are more mature than those of sieve size 5 in samples A and C. The organoleptic grade of sample H also indicates a more mature sample of peas.

TABLE 2.—*Calcium and phosphorus content and calcium-phosphorus ratio of the skins of different sieve sizes of 2 varieties of canned peas*

[Lots and sieve sizes correspond to those of table 1]

Sample	Variety	Phosphorus in sieve size—			Calcium in sieve size—			Ca/P ratio×100 for sieve size—		
		3	4	5	3	4	5	3	4	5
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*			
B	Perfection	0.179	0.172	0.150	-------	0.705	0.946	-------	409.9	630.7
C	Lot 100	.196	.176	.146	0.678	.620	.715	345.9	352.3	489.7
D	Perfection	.145	.130	.129	.579	.663	.670	399.3	510.0	519.4
E	Lot 100	.180	.145	.106	.599	.595	.704	332.8	410.3	664.2
H	do	.124	.101	.086	.542	.526	.573	437.1	520.8	666.3
I	do	.184	.148	.117	.477	.595	.542	259.2	402.0	463.2
J	do	.156	.137	.103	.537	.546	.597	344.2	398.5	579.6

Table 2 shows the calcium and phosphorus analyses of the pea skins of the canned product from the same lots shown in table 1 with corresponding numbers and sieve sizes. The percentage of calcium in the pea skin is increased by the canning process. This agrees with the finding of Sayre, Willaman, and Kertesz, who showed an increase in the calcium content and a decrease in the ash content of the canned product. The phosphorus content of the pea skin decreases with canning. This loss of phosphorus may be accounted for in the liquor, but the reason for the increase of calcium in the skins of the canned product over that in the peas is not clear.

An increase in the calcium-phosphorus ratio of the more mature pea over that of the less mature pea is shown in table 2, but whether the calcium-phosphorus ratio in the canned pea is as good an index of maturity as it is in the raw pea is questionable because of the loss of phosphorus in the liquor and the increase of calcium in the skins of the canned product.

The results in table 3 show that the calcium-phosphorus ratio of the mature pea skins is much higher than the calcium-phosphorus ratio of the skins of either the raw canning peas or the canned product. In table 2 the highest calcium-phosphorus ratio shown for the canned product is 666.3, which is much lower than any of the ratios from the skins of the mature peas shown in table 3. In view of these wide differences, the calcium-phosphorus ratio may serve as a means of distinguishing the raw canned peas from the soaker canned peas.[4]

[4] Soaker canned peas are mature seed peas that have been presoaked and canned.

TABLE 3.—*Calcium and phosphorus content and calcium-phosphorus ratio of the skins of mature seed peas*[1]

Variety	Calcium	Phosphorus	Ca/P×100
	Percent	*Percent*	
Blue Bell	1.00	0.089	1,124
Alaska	1.18	.084	1,405
Kaiser	1.18	.106	1,113
White Canada	1.04	.083	1,253
Earliest of All	.97	.073	1,329

[1] Except for the variety Earliest of All, the data in this table were taken from an Idaho station bulletin (*1*) and recalculated to the elemental calcium and phosphorus.

Since the increase in calcium content and the decrease in phosphorus content of the pea skins vary inversely with maturity, the ratio of these two elements should provide a good method for the determination of maturity and quality of the raw peas for canning purposes. The use of the calcium-phosphorus ratio eliminates errors arising from variations in the moisture content of the pea.

However, several comparisons must be made with well-known tests for quality before a definite calcium-phosphorus ratio can be used as an index to quality.

SUMMARY

Data have been presented which show that there is a marked variation in the calcium and phosphorus content of the pea skin at different stages of maturity. Analyses of the skins show that the calcium content and the calcium-phosphorus ratio increase and the phosphorus content decreases with maturity.

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